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Transport of Fatty Acids

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THE ORGANIZATION of the higher forms of life into specialized organs and tissues, including a circulatory system which serves to interrelate their chemical activities, requires that a wide variety of substances be transported from one part of the organism to another. It is the purpose of this communication to review investigations into the problems of transport of fatty acids. These compounds, together with the sterols, the much less common acyclic fatty alcohols and traces of hydrocarbons, make up the lipids, the transport of which presents a peculiar problem to the organism because of their limited solubility in water. For many years, the study of lipid transport has lagged far behind investigations of the transport of water-soluble substances. In the short time, however, since adequate techniques have become available for studying lipids 'in motion,' this field has developed almost explosively.

This review will be limited to a discussion of the transport of fatty acids in the mammal. The complex lipids, such as triglycerides and phospholipids and sterol esters, will be dealt with only in the context of their participation in the movement of fatty acids. It is also proposed to limit the use of the term 'transport' to imply net transport, or to biochemical processes involving the removal of a substance from one tissue or area and its delivery to another. Mere existence in the circulating blood is not considered evidence that a substance is being transported; it might equally well be fulfilling a vehicular function. Consideration of the familiar example of hemoglobin and oxygen will make the differentiation clear, particularly if we somewhat arbitrarily add to the definition of 'net transport' the qualification that the biochemical process under scrutiny appears to serve a useful role in the economy of the organism. Without this qualification, one might say, for example, that a function of the erythrocyte is to transport hemoglobin from bone marrow to spleen.

The fatty acids are recognized as being of importance to living organisms in two primary roles. Firstly, they serve as substrates for oxidative catabolism and production of energy, and their high energy equivalence per unit weight makes them important in the storage of energy. Secondly, complex lipids of which the fatty acids are essential components serve as structural components of cells and of sub-cellular organelles. Their insolubility in aqueous media makes the complex lipids suitable for this function. In spite of the rapidity with which growth, repair and turnover of structural elements is now believed to occur, it is the need to supply oxidizable fatty acids to functioning tissue cells that imposes the major demand on fatty acid transport mechanisms. Also, it is essential that this phase of fatty acid transport be related in a mutually complementary fashion to the metabolism of carbohydrate, which constitutes the other principal source of energy. It is proposed to review first the physical state and chemical composition of the fatty acids in extracellular fluid. This will include a fairly comprehensive review of the lipoproteins, since much recent information concerning them has not been assembled in the context of their vehicular

role in fatty acid transport. The literature pertaining to turnover and metabolism of unesterified and esterified fatty acids in the extracellular fluid will then be reviewed with as much conceptual organization as the available data permit.

The present review being limited to a relatively narrow aspect of fat metabolism, reference will be made to a number of recent reviews of related subjects. Three of these, dealing with important subjects that have not been covered, include a recent review of fatty acid synthesis and oxidation and metabolism of phospholipid by Kennedy (209), a review of intestinal absorption of lipid by Bergström and Borgström (26), and a review of the biochemical behavior of adipose tissue by Shapiro (324). The reader is asked to forgive the number of references to work which has not yet been published. An attempt has been made to limit them, but, in many areas, they cover information too helpful to ignore.

Unesterified Fatty Acids in Extracellular Fluid

From the chemical point of view, the simplest of the plasma fatty acid compounds is the fraction known as unesterified fatty acids (UFA). The terms nonesterified fatty acid (NEFA) and free fatty acid (FFA) have also been used to describe the same fraction. The former is obviously a true synonym, whereas the latter is less accurate, as will be explained below. The unesterified fatty acids are those that exist in plasma primarily as anions, though at the pH of plasma, one would expect to find somewhat less than 1 per cent of the fatty acid in the undissociated state (252). As the name implies, the carboxyl group of the fatty acids of this fraction is not involved in the formation of an ester bond.

The earliest experimental evidence for the presence of unesterified fatty acids in plasma is that of Szent-Györgyi and Tominaga (347) published in 1924. Although their report was not confirmed by other workers at the time, it appears in the light of more recent studies that the UFA concentrations found were in the correct range. Some years later, Kelsey and Longenecker (208) described the recovery of free fatty acids from their fractionation of the lipids extracted from bovine plasma. The quantity found was equivalent to 0.95 mEq/l. of plasma. Their results are open to question, however, for as they themselves observed, the time required for the processing of so large a quantity of plasma (90 liters) might have allowed hydrolytic processes to proceed *in vitro*, with the artifactual production of excessive quantities of UFA. Davis (94) noted the presence of UFA in human plasma, and unesterified fatty acids were uniformly present in the albumin fractions isolated from human plasma by Cohn and his collaborators (89).

In recent years, several groups of investigators have become interested in the physiologic significance of plasma unesterified fatty acids. Methods for the estimation of the long-chain unesterified fatty acid content of small plasma samples have been developed in several laboratories; these procedures are rapid and minimize the possibility of artifactual hydrolytic processes occurring *in vitro*. All interested investigators now agree that UFA is present in the plasma of man and the common laboratory mammals under physiologic circumstances, and all find concentrations in the range of 0.2–2.0 mEq/l.

A problem exists with respect to analytic methods for the determination of UFA concentrations. A number of methods for the determination of UFA concentrations have been proposed (94, 99, 167, 175), most of which are similar in that the basic process is titration of the fatty acids after their extraction from plasma. One method, that of Coleman and Middlebrook (91), employs a colorimetric principle.

None of the existing methods has been evaluated critically, so that no standard exists for the testing of new analytic procedures as they may be proposed. Both Dole (99) and Gordon (167) have given some evidence for the reliability of their methods, but in each case, the justification of the method consists of showing that fatty acids added to plasma are recovered, and that a variety of other substances are not. Such an approach does not prove unequivocally that the titratable acids already in the plasma are identical to those added. Indeed, the average normal values reported by Dole appear rather higher than those observed by Gordon, suggesting that their methods are not equivalent. Differences between these methods, both of which have been shown to afford good recovery of long-chain fatty acids added to plasma *in vitro*, would suggest either that one method (Dole's) is more successful in recovering short-chain acids as well, or that it also recovers and titrates more acids that are not of the fatty acid series. Further studies designed to evaluate analytic methods, and to identify positively the acids extracted from normal plasma, are urgently needed. Another need, particularly important for the application of knowledge concerning unesterified fatty acid metabolism to the practice of medicine, is for a suitably reliable and very rapid bedside analytic method. The method of Dole approaches this ideal more closely than any other of the existing proven methods, but still requires too much time, and too much specialized microchemical equipment, to be easily applicable to the management of certain urgent conditions, such as diabetic coma, in which the serial determination of UFA concentrations might be of practical usefulness.

Composition of Plasma Unesterified Fatty Acids. Intimately related to this need for better analytic methods is the need for knowledge of the composition of the fatty acids present in the UFA fraction. In their study of bovine plasma lipids, Kelsey and Longenecker (208) tabulated the fatty acids found in the UFA fraction. They observed primarily palmitic, oleic and linoleic acids, with small amounts of stearic, myristic and arachidic. Information on the composition of human UFA is limited to one study by Dole (100), in which by silicic acid column chromatography palmitic and oleic acids were identified, and to Dole's references (101–103) to observations, as yet unpublished, that he and James have made by applying gas phase chromatography to the separation and identification of the fatty acids occurring in unesterified form. In these studies, the fatty acids encountered were noted to be similar to those occurring in the plasma triglycerides or in the depot fat. Acids which have been identified include the saturated acids from C_{10} to C_{18}, and also oleic, palmitoleic, linoleic and longer chain polyethenoic acids (103). No acids peculiar to the UFA fraction have been encountered, a finding that makes recovery experiments for the evaluation of analytic procedures appear more valid.

Fatty acids of short chain length occur in plasma, particularly in that of ruminants. The methods for the estimation of these acids (steam distillation of an acidified deproteinized plasma filtrate) are such that fatty acids incorporated into glycerides or strongly bound to proteins would not be recovered. Annison (14–16) tabulates the volatile fatty acids encountered in the blood of sheep, and states that he found formate in the blood of several non-ruminant species, including man. Formate was present in erythrocytes at a higher concentration than in plasma. Acetate has also been detected in the plasma of human subjects (129). These short-chain fatty acids appear to differ from the long-chain UFA with respect to origin and metabolic fate; hence, it is desirable that they not be included with the long-chain UFA in analytic procedures used in physiologic experimentation. It is stated that fatty acids of

short-chain length are not determined by the method of Coleman and Middlebrook (91), and none would be expected to be titrated in the method of Gordon (167) in which glacial acetic acid is used as an extracting solvent.

Interactions of Unesterified Fatty Acids With Proteins. That fatty acid anions do not exist free in plasma was first shown by du Noüy (228), who observed that the surface tension lowering effect, characteristic of fatty acid anions, was lost when soap solutions were added to serum. He inferred that there was an interaction between the soap molecules and the proteins of serum. Teresi and Luck (349) in 1951 made use of the method of dialysis equilibrium to obtain the first quantitative analysis of the interaction of the salts of 2- to 8-carbon fatty acids with crystalline bovine serum albumin. Unfortunately, the preparations of serum albumin available to them were of a type that usually already contains some bound unesterified fatty acid (162). Such fatty acids already present on the protein, especially if they were of longer chain length and more firmly bound than the acids under study, would cause an error in the direction of underestimation of the number of binding sites and of the strength of the interaction. The results of these experiments were interpreted as showing the presence on albumin of two classes of binding sites. The first class, four to five in number, had a moderate affinity for the fatty acids tested, and the second class, numbering about 27, had a lesser affinity. The binding constants increased with increasing length of the hydrocarbon chain of the fatty acid. These results are strikingly similar to those of Goodman (163; cf. table 1), with the exception that Teresi and Luck did not observe the presence of a few sites with very high affinity for fatty acid anions. It is not unlikely that these sites were already occupied by long-chain fatty acids in the preparation of crystalline albumin which served as their starting material.

Recent studies by Goodman (163–165) have yielded data sufficient for a quantitative treatment of the binding of fatty acids by several of the plasma proteins, and also by erythrocytes. He has prepared human serum albumin almost devoid of fatty acids for a starting material (162). To suitable amounts of this albumin, dissolved in a buffer of physiologic pH and ionic strength, were individually added six different fatty acids, each over a wide range of concentrations. By determining the concentration of free (unbound) fatty acid in each solution, he obtained data relating the average number of molecules of fatty acid bound by each molecule of albumin to the concentration of free fatty acid in the system. Treatment of these data by the methods developed by Scatchard (315–317) allowed determination of the number of binding sites on each albumin molecule, and of the apparent association constant for each class of sites. The treatment is based on the assumptions that each albumin molecule bears a discrete number of sites to which fatty acids are bound, that the sites are

TABLE 1.* APPARENT ASSOCIATION CONSTANTS FOR INTERACTION OF HUMAN SERUM ALBUMIN WITH FATTY ACID ANIONS AT pH 7.45, IONIC STRENGTH 0.160, 23°C WITH THREE CLASSES OF BINDING SITES, $n_1 = 2$, $n_2 = 5$, AND $n_3 = 20$

FATTY ACID ANION	k'_1	k'_2	k'_3
Laurate	1.6×10^6	2.4×10^5	6×10^2
Myristate	4.0×10^6	1.4×10^6	2×10^2
Palmitate	6.0×10^7	3.0×10^6	1×10^3
Stearate	8.0×10^7	8.0×10^5	1×10^3
Oleate	1.1×10^8	4.0×10^6	1×10^3
Linoleate	1.3×10^7	2.5×10^6	2.5×10^3

* After Goodman, D. S. (163); reprinted with the permission of the Journal of the American Chemical Society.

independent (in other words, the binding of a fatty acid to one site does not alter the intrinsic association constants of the other sites on the same molecule), and that the interaction of any one site with a fatty acid is a simple association-dissociation equilibrium that obeys the law of mass action and can be described quantitatively by an association constant (reciprocal of a dissociation constant). The constants may be different for different fatty acids, and, in addition, it is not to be anticipated that all the sites of the albumin molecule will be identical. Goodman has found that each albumin molecule bears two sites with very high affinity for fatty acids, five with a lesser affinity, and a large number (approximately 20) with very much less ability to bind fatty acids. The apparent association constants at pH 7.4 and ionic strength 0.16 for these three classes of sites on human serum albumin for the fatty acids studied are given in table 1. It should be noted that this quantitative study does not set a limit to the 'binding capacity' of albumin for fatty acids. Rather, the number of moles bound increases with increasing free fatty acid concentration, and the only practical limit is set by the solubility of the fatty acid itself in an aqueous system. The binding capacity of albumin with respect to some other biological or chemical system will be the number of moles of fatty acid that each mole of albumin can bind without the concentration of free fatty acid exceeding some threshold set by the phenomena under observation. Thus, Davis and Dubos (95) noted that albumin in their culture media could bind 5 moles of oleate before inhibition of growth of *M. tuberculosis* occurred. Likewise, Gordon *et al.* (172) observed that approximately seven moles of oleate could be bound per mole of albumin before free oleate produced complete inhibition of the hydrolysis of coconut oil by lipoprotein lipase ('clearing factor').

This knowledge of the relation of free fatty acid concentration to number of moles bound by albumin has made it possible to use albumin as an indicator in studying the binding of fatty acids by other blood components. Gordon (166), Laurell (225), and Mora, *et al.* (258) had noted that oleate was bound by lipoproteins as well as by albumin, but the electrophoretic data were not of sufficient precision to allow quantitative treatment. By equilibrating mixtures of albumin, Low-Density[1] lipoproteins, and fatty acids, under physiologic conditions of pH and ionic strength Goodman and Shafrir (165) have obtained data that permit a quantitative evaluation of the interaction of these lipoproteins with five of the more common fatty acids on the basis of numbers of binding sites and apparent association constants. However, these figures are necessarily less precise than those describing the interaction of fatty acids with albumin. This is because any errors incurred in the first study affect the later ones based thereon, and because the lipoproteins are less stable and represent less reproducible preparations than does albumin. The investigation of the binding of UFA by High-Density[1] lipoproteins presents greater difficulties, since no method is known for the separation of the lipoproteins from albumin after

[1] The introduction of capital letters in the names of the two groups of lipoproteins will be arbitrarily done here so that reference may be made throughout this review to lipoproteins that are comparable to them. Methods for isolating lipoproteins other than ultracentrifugation give rise to their own terminology and may yield lipoprotein isolates which do not strictly represent the density limits used by Gofman. For example, a low density lipoprotein is arbitrarily defined as a lipoprotein which floats to the top of the ultracentrifuge tube in the Spinco Model L at a solution density of d_4^{26} 1.063 when centrifuged for 13 hours at 40,000 r.p.m. in the 40.3 rotor (193, 235). It has been proposed (237) that all lipoproteins be designated in terms of the method of isolation, and this rule will be occasionally abandoned only in the interest of continuity.

equilibration that is not likely to disturb the very equilibrium that one wishes to measure. Erythrocytes, however, are easily separated from albumin, and the partition of palmitate between red cells and albumin has been measured by Goodman (164).

The binding of palmitate to erythrocytes was interpreted in terms of the presence on the cells of a large number of binding sites (one per 420 square Ångstrom units of surface area, or 3.2×10^7 per individual cell) having an apparent association constant under physiologic conditions of 2.2×10^6. These binding sites were shown to be present on the stromata after hemolysis of the cells in hypotonic media. The lipoprotein fraction of density 1.019 to 1.063, on the other hand, was shown to bear two classes of receptor sites. The first class, present in small numbers, had an apparent association constant for palmitate of 3×10^6, and the second class, of which there are much larger numbers, had an apparent association constant of 1.7×10^5. Lipoprotein of density below 1.019 behaved in similar fashion but the constants were slightly higher. Laurate, stearate, oleate and linoleate were also studied, and the association constants for each acid determined. In normal human blood, the relative concentrations of Low-Density lipoprotein, erythrocytes, albumin and unesterified fatty acids are such that (for palmitate), one would expect to find approximately 1 per cent bound to the red cells, 0.5 per cent to the Low-Density lipoproteins, and 0.01 per cent unbound. The fraction bound to High-Density lipoproteins is as yet unknown, and the remainder would be bound to albumin. The relationships for the other fatty acids studied would be quite similar to those predicted for palmitate, and these acids constitute over three fourths of the circulating UFA fraction according to the best information available at present. Fatty acids of unusual chain length or configuration might be partitioned differently, however, and it is not unreasonable to suppose that lipoproteins or red cells may bind the greater share of some minor components of the UFA fraction.

Goodman and Shafrir observed that the partition of palmitate between albumin and Low-Density lipoproteins was altered by changes in the pH or ionic strength of the medium. The addition of salts, in particular, was effective in displacing the equilibrium toward greater relative binding by lipoproteins. For this reason, studies of the partition of UFA which are performed by analysis of plasma fractions separated in media of high ionic strength (157, 298, 323) are not satisfactory for quantitative determination of the distribution of the unesterified fatty acids among the various plasma proteins *in vivo*.

The details of the mechanism responsible for the binding of UFA to albumin are as yet unknown. It is presumed that the unesterified fatty acids bound to albumin are in the dissociated form, though this presumption has not been rigorously proven. The electrophoretic mobility of albumin has been noted (87) to increase when fatty acid anions are added to the buffer, showing that the albumin-fatty acid complex is more negative than is albumin alone. Further, it has been demonstrated (279) that albumin has a strong affinity for the alkyl sulfonic acids, whose dissociation constants are so high that they could not reasonably be expected to be present in neutral solution in any but anionic form. Goodman (163) has discussed similarities between the results of his studies and those of Scatchard, Coleman and Shen (316) on the binding of chloride, iodide, thiocyanate and trichloroacetate (all anions). Finally, it would be extraordinarily difficult to conceive of any molecular basis for such strong interactions as those observed in the UFA-albumin system without invoking an electrostatic factor derived from the mutual attraction of the carboxylate anion and some positively charged group on the surface of the albumin molecule. As

yet, however, no data are available from which the configuration of the UFA binding sites on albumin can be deduced. Any proposed model must account for the requirement for an anionic group, for a measure of selectivity with regard to the length and unsaturation of the hydrocarbon moiety (cf. table 1), and for facile dissociation of the bound fatty acids.

Kinetic aspects of the binding of fatty acid ions to albumin and other substances have not as yet been investigated. As will be seen, the rate of transfer of unesterified fatty acids *in vivo* is considerable, the average fatty acid molecule remaining in the plasma for only a few minutes at a time. No binding protein, however strong its affinity for fatty acids *in vitro*, could fulfil the necessary function *in vivo* unless it can bind and dissociate fatty acid ions at a rate that is rapid relative to the known turnover of the fatty acids themselves. In unpublished experiments in our own laboratory, it has been shown that the rate of binding of fatty acid ions by human serum albumin is at least of the correct order of magnitude. The addition of sodium oleate solution to the colored complex formed by albumin and the sodium salt of 2-[4′-hydroxybenzeneazo-] benzoic acid causes the displacement of the organic dye from the albumin, resulting in an easily visible color change. This color change appears complete as soon as two suitable solutions are mixed at room temperature. It is therefore apparent that precise measurement of the rates of such binding reactions will be a task of some complexity. However, in all probability the reaction rates are so rapid that the equilibrium relationships observed *in vitro* may be applied without risk of major errors to *in vivo* systems in which transfer processes are occurring.

Esterified Fatty Acids in Extracellular Fluid

Over 90 per cent of the fatty acids found in plasma are present as esters of glycerol or more complex alcohols such as sphingosine and cholesterol. These esters depend upon combinations with certain of the plasma proteins other than albumin for solubility in the aqueous extracellular fluid. Of about 300 to 400 milligrams per cent (10 to 15 mEq/l.) esterified fatty acids found in the plasma of normal humans in the postprandial state, triglycerides and phospholipids account for approximately 40 per cent each, and the remainder are in cholesterol esters. Small amounts have been reported to occur as di- and monoglycerides (82, 83), as cerebrosides (118, 210), and as acetals (178).

The Lipoproteins. It is now well established that these lipid-protein combinations form a variety of lipoprotein molecules which have certain chemical specificity and other unique properties which warrant their consideration as distinct biochemical entities. Several reviews have recently appeared which describe in detail some of the historical background, clinical aspects and techniques utilized to isolate the lipoproteins (108, 132, 157, 272, 274), and their description here will be oriented so as to emphasize our present understanding of their role and interrelationships in the transport of fatty acids. While most of the detailed information concerning the lipoproteins has been obtained from studies utilizing plasma, those studies which have been made comparing the lipoproteins of plasma and lymph (93, 278, 303–305), make it fairly certain that all extracellular fluid contains a single lipoprotein system, with perhaps the only major difference being concentration gradients for certain of the lipoprotein species.

The first lipoprotein was obtained from horse serum by Machebouef in 1929 (247), when he used salt precipitation to isolate what now would be considered a High Density lipoprotein. Methods for isolating or characterizing lipoproteins, based

on their electrophoretic migration (37, 104, 121, 221, 309, 341), differences in density (158, 235), or solubility in cold ethanol-buffer mixtures (88, 90), were next developed, and have since been refined in a variety of ways.

As a result of this intensive effort, it has now been adequately demonstrated that there are at least three major groups of lipoproteins present in the plasma of man and all mammals so far studied. These are: *1*) High Density[2] lipoproteins, $\rho > 1.063$, also referred to as the α_1-lipoproteins by virtue of their electrophoretic mobility, which include the HDL_2, HDL_3 and part of the HDL_1 classes of deLalla, Elliott and Gofman (96), and the —S 0–10 classes isolated by the method of Lewis and Page (232); *2*) Low Density[2] lipoproteins, $\rho < 1.063$, which migrate on paper and free electrophoresis as β-lipoproteins and on starch as both β- and α_2-lipoproteins (222), and include the classes S_f 0 to about 400, according to the method of Lindgren, Elliott and Gofman (235), and —S > 10 classes according to the method of Lewis and Page (232); and *3*) chylomicrons, which are emulsified particles of lipid, normally present only during fat absorption. There is no fine line of demarcation between the smallest chylomicron particles and the lightest of Low Density lipoproteins; for this reason, and because the chylomicrons are now known to be associated with significant amounts of protein, they will be considered here as lipoproteins.

The lipoproteins of lymph and plasma range in density from about 0.9 to 1.2 grams per milliliter, and the best present methods for measuring, isolating, and partially purifying them utilize differences in their density to achieve segregation in high centrifugal fields. These techniques were first developed by Gofman and his colleagues (96, 158, 235), and several modifications (190, 195) employing segregation between arbitrary density bands in the preparative ultracentrifuge have outstanding usefulness in separating lipoproteins in quantities sufficient for chemical analysis. One very useful procedure recently published is that of Oncley, Walton and Cornwell (276), in which dextran sulfate is used to precipitate the Low Density lipoproteins alone from whole serum. They may then be subfractionated by ultracentrifugation in a gradient tube. The techniques of electrophoresis and Cohn fractionation also have practical but limited usefulness, and other cruder separations have been accomplished by techniques using precipitation of Low Density molecules with another high molecular weight polymer, heparin (77), or separation on glass beads (80) or other adsorbents such as calcium oxalate (313). Quantitative characterization of lipoprotein aggregates obtained in the preparative ultracentrifuge may be obtained by recentrifugation in the analytical ultracentrifuge. By optical methods, species or 'classes' of lipoproteins can be observed floating at different rates. The boundaries of these upward migrations, appropriately standardized, can be used to measure the concentrations of the various classes (96, 232, 235). Using these boundary measurements, Gofman has broken the Low Density lipoproteins of $\rho < 1.063$ into various classes which are assigned flotation rates measured in Svedberg units of flotation (the S_f unit, 10^{-13} cm gm sec^{-1} $dyne^{-1}$). The migrations of lipoproteins of S_f 0 to 400 are slow enough for most accurate quantitation, grouped in several classes for convenience, although faster moving boundaries, including the chylomicrons (assigned an S_f of about 4×10^4 to 10^5) (235) are also present. The S_f terminology is not to be confused with the negative Svedberg notation, —S, sometimes used by other workers. For example, Lewis and Page (232) apply the designation —S 10 or greater to those lipoproteins floating at $\rho < 1.063$.

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[2] See footnote 1, page 589.

High Density Lipoproteins. These lipoproteins, $1.063 < \rho < 1.21$, (α_1-lipoproteins) have been separated in the analytical ultracentrifuge into several species by deLalla, Gofman and Elliott (96), who describe HDL_1, HDL_2 and HDL_3 classes with hydrated densities of approximately 1.05, 1.075, 1.145 gm/ml, respectively. Because its density overlaps into the Low Density lipoprotein range, much of the HDL_1 class may be isolated with the heavier Low Density lipoproteins. The composition of the High Density lipoproteins reflects their position at the heavy end of the lipoprotein density 'spectrum,' which, in turn, is characterized by steady decline in the proportion of protein and a converse increase in the proportion of triglycerides as the molecules decrease in density. An average composition of the major High Density lipoproteins in human plasma includes about 50% (by weight) of protein, 25% phospholipid, 20% cholesterol, most of which is esterified, and less than 10% triglyceride (65, 157). Thus, a quarter of the fatty acids found in these lipoproteins are in triglycerides and the rest are about equally distributed between phospholipid and cholesterol esters. As mentioned above, a small amount of unesterified fatty acid might also be present. The smallest of the lipoproteins in size, a High Density lipoprotein, or α_1-lipoprotein isolated from Cohn Fraction IV-1 (90) was estimated by Oncley, Scatchard and Brown (275) to be an ellipsoid molecule with axes of 50 and 300 Å, and to have an anhydrous molecular weight of about 200,000. Hazelwood (193) has recently reported some molecular weights on HDL_2 and HDL_3 lipoproteins isolated by ultracentrifugation (96). Using an approach which does not depend upon assumptions about molecular shape, he found hydrated molecular weights of 1.75×10^6 and 4×10^6 for the HDL_3 and HDL_2, respectively. Assuming prolate ellipsoids, the dimensions of these macromolecules would have been 40×130 and 90×365 Å, respectively. The physical constants obtained for these lipoproteins by different laboratories may not be strictly compared, due to the different methods of isolation employed.

The full significance of the High Density lipoproteins in the transport and metabolism of fatty acids is not known. However, in many animals, most of the lipid present in the postabsorptive state is carried in High Density lipoproteins (190). Much the same situation appears to exist in the very young human, but in the adult a larger fraction of the total plasma lipid is found in the Low Density lipoproteins (157, 290, 331). This is due more to a steady rise in the Low Density lipoprotein concentrations than to the small absolute decrease in High Density concentrations which occurs with age (157). Changes in the concentrations of the HDL_1, HDL_2, and HDL_3 lipoproteins have also been demonstrated to occur independently of each other and of the major Low Density lipoproteins (157). The relationship of High Density lipoproteins to the remaining lipoproteins will be developed further below.

Low Density Lipoproteins. These lipoproteins ($\rho < 1.063$) represent a broad and possibly inhomogeneous group, which, by virtue of their greater percentage content of lipid and greater lability in concentration, may have even greater metabolic importance than the major High Density lipoproteins. As has been mentioned, the best method of separating and measuring the largest number of species within the Low Density group consists in the preliminary isolation of all lipoproteins of $\rho < 1.063$ and recentrifugation of this aggregate in the analytical ultracentrifuge (235).

The Low Density molecules of $1.019 < \rho < 1.063$ correspond roughly to S_f 0–12, and migrate electrophoretically with the β-globulins, hence the name β-lipoproteins. The Low Density lipoproteins of $S_f > 12$ and of $\rho < 1.019$ migrate on starch with the α_2-globulins (222). All of the Low Density classes are contained in Fraction

III in the alcohol precipitation procedures of Cohn and collaborators (88) and all are precipitated by dextran sulphate according to the method of Oncley *et al.* (276). The composition of the Low Density lipoproteins is characterized by increasing triglyceride and decreasing phospholipid, cholesterol and protein as the density of the molecules decreases. Hence, about one-quarter of the fatty acids present in the class S_f 0–10, for example, are in triglycerides, and the rest are distributed between cholesterol esters and phospholipid (65). While this fractional distribution is similar to that in the major High Density molecules, the ratio of cholesterol to phospholipid is reversed, and is greater than unity, in the S_f 0–10 class. As the S_f values rise and the density progressively decreases, triglycerides become the predominant ester form of the fatty acids and make up more of the total composition of the lipoproteins. Nearly all of the carotene in plasma is carried by Low Density lipoprotein (176, 178). The molecular weights of all the Low Density lipoproteins are probably greater than 10^6, and several estimates of size and shape of the molecules have appeared. Oncley, Gurd and Melin (273) estimated a β-lipoprotein they isolated from Cohn Fraction III-0 to have a hydrous molecular weight of 1.3×10^6, and to be spherical in shape with a diameter of about 185 Å. Bjorklund and Katz (35), studying molecules of S_f 5 to 8 by light-scattering and ultracentrifugation, found molecular weights of 2.8 to 3.1×10^6, and suggested the molecules had the shape of ellipsoids with small axial ratios. Their data suggested that attenuated or coiled shapes for these molecules were very unlikely. Prendergast and Teague (287) and Beischer (24) have examined by electron microscopy Low Density lipoproteins corresponding to S_f 10–30, and found particle diameters varying from 100 to 400 Å, usually in two distinct maxima of distribution, indicating that within an S_f class such as S_f 10–20 there is a considerable spectrum of particle size. The shape of the particles they observed was usually spherical; some were ellipsoidal. Beischer also noticed considerable difference in staining of the particles with osmic acid, emphasizing possible important differences in composition within given density classes.

Chylomicrons. 'Chylomicron' is the term first applied by Gage in 1920 (147) to the large particles (0.5–1.5 μ) observed on dark-field microscopy of thoracic duct lymph or plasma obtained during fat absorption. In a most significant article published in 1924 (148), he and Fish reviewed the history of these particles, from the time they were first observed in the microscope in the eighteenth century to the establishment by the authors that they were definitely particles of fat being transported from the intestine to the blood. Under certain conditions, such as diabetic acidosis (10, 47), the nephrotic syndrome and idiopathic hyperlipemia (10), chylomicrons are also responsible for most of the lactescence seen in plasma in the post-prandial state. With the yielding of the microscope to the ultracentrifuge in the study of fat transport, the definition of a chylomicron has become less, rather than more, simplified. Since the chylomicrons float rapidly in the centrifuge, the decision where the chylomicrons stop and the very light Low Density lipoproteins begin has become arbitrary. Hence, qualifying adjectives such as 'white chylomicrons' (198) for the material of lightest density, or 'lipomicrons' (251) for lipoproteins rich in triglycerides, which float rapidly on centrifugation but which are smaller in particle size than 0.5 μ, have appeared in the literature. At present, the chylomicrons may best be defined operationally as the material which is concentrated at the top of the tube when lactescent chyle or plasma is layered under media at density 1.006 and centrifuged for a few minutes at high speeds. Lindgren, Gofman, and Elliott have suggested 9500 *g* for 10 minutes (235), and workers in this laboratory (63, 191, 303–306) have used speeds

up to 100,000 *g* for 30 minutes. The major difference detected between these preparations is a small increase in the amount of protein obtained with the higher speeds (63) and it is assumed that the lower the speed and the shorter the time of centrifugation, the 'purer' will be the chylomicron fraction. When chylomicrons obtained from lymph over the range of conditions suggested above are resuspended and washed repeatedly (3–8 times) by centrifugation, the final product will be readily dispersible into a fine emulsion which will contain 85–90 per cent triglyceride and small amounts of phospholipid and cholesterol, usually in a ratio of about 2:1 (63, 191, 223). Very small amounts of unesterified fatty acids may also be present. The emulsions will contain from 0.2 to 1.0 per cent protein (63, 303–306). Robinson (297) has reported isolating some chylomicrons without detectable protein, but with methods more sensitive than the micro-Kjeldahl methods he employed, tightly-bound peptide is consistently obtained (63, 191, 223, 303–306). Furthermore, the delipidated protein from chylomicrons obtained at very low speeds for short times is the same electrophoretically as that from chylomicrons obtained at 100,000 *g* for 30 minutes (305). Since chylomicrons apparently carry a negative charge, they can be flocculated by protamine (75) and by toluidine blue (223), each a polyfunctional cation. Laurell has found the latter useful as a preparative method, since chylomicrons clumped by toluidine blue can be freed of the dye by dialysis and readily resuspended. Many workers have observed that lymph chylomicrons may undergo some change when entering plasma. Frazer (131) proposed a difference on the basis of electrophoretic mobility measurements, the lymph chylomicrons migrating with albumin on free electrophoresis and the plasma chylomicrons migrating with alpha or beta globulin. This was subsequently confirmed by Swahn (342) and by Laurell (224) who observed that after incubation with plasma, lymph chylomicrons migrated with the α_2-globulins. While there is no reason to doubt the validity of these observations, and other evidence supporting them will be mentioned below, it should be pointed out that electrophoresis of chylomicrons is generally difficult and cannot be used as a preparative method. Zone electrophoresis of washed plasma chylomicrons is usually unsatisfactory, since they may either remain at the origin or move with the α_2-globulins. Moving boundary electrophoresis of isolated chylomicrons is also unsatisfactory, due to flotation; in whole lipemic serum their movement may be determined by observing the boundary of turbidity. Under these conditions their mobility is that of the α_2-globulins (222, 269, 342). Laurell (224) also noted that more toluidine blue was required to flocculate plasma chylomicrons. Plasma chylomicrons isolated under the same conditions as those employed for lymph also may be less stable during repeated washing (364).

Chemical Interrelationships of Lipoproteins. Understanding of the relationships of the various groups of lipoproteins to each other is far from complete. One problem is the isolation of 'pure species' of lipoproteins, which, in turn, is related to the need for means of identifying lipoprotein molecules which may be even more specific than their lipid composition, density or molecular weights. The recent applications of immunological and chemical techniques to characterize the peptide residues of the lipoproteins show considerable promise in this regard. Avigan, Redfield, and Steinberg (19), Shore (328), and Brown *et al.* (71) have determined the principal N-terminal amino acid residues of the peptide chains isolated with lipoproteins within certain densities, and Rodbell (303) has recently extended these analyses to lymph chylomicrons and the entire plasma 'spectrum.' In addition, Shore has also analyzed several fractions for their C-terminal residues, and he and Brown and co-workers

(71) have reported that the amino acid composition of the peptides in both High and Low Density lipoproteins is quite similar. Scanu, Lewis and Bumpus have also studied the protein of the major High Density lipoproteins (314).

The delipidated peptide residues of the High Density lipoproteins have been estimated to have hydrous molecular weights from 75,000 (314) to $1-2 \times 10^5$ (328), varying with the particular fraction analyzed. These proteins all appear to contain N-terminal aspartic acid (19, 71, 303, 314, 328), and C-terminal threonine (328). Shore (328) has suggested that a lipoprotein corresponding to HDL_2 ($\rho = 1.093$) may contain two of the peptide chains found in the HDL_3 class. The nature of the peptide residues which may be found in the Low Density lipoproteins is still incompletely resolved. Workers using immunochemical techniques have reported that the S_f classes <10 and >10 were, on the one hand (154), heterogeneous, and on the other hand (6, 155, 219, 231), indistinguishable immunologically. All these studies, and others, (312) have agreed that the major High and Low Density lipoproteins are not immunochemically identical, although Aladjem, Lieberman and Gofman felt that HDL_2 lipoprotein may have some antigenic properties in common with some Low Density lipoprotein (6). Major agreement exists that the S_f 0 to about S_f 20 classes contain peptide residues which have principally N-terminal glutamic acid (19, 71, 303, 328). However, the difficulties of obtaining sufficient protein from the very light Low Density classes have discouraged full characterization of this group. Recently Rodbell (303) has reported analyses of the N-terminal acids on the protein obtained from human lipoproteins isolated over the full density 'spectrum.' While this work must be extended, from his data it would appear that the peptide residues of most of the Low Density lipoproteins of about S_f 100 and greater may be different from those of lower S_f, in that they contain almost no N-terminal glutamic acid, but rather serine and threonine. These two amino acids are also N-terminal in much of the protein which is isolated with the chylomicrons (303–306 and fig. 1). Thus, there remains the possibility that there may be several types of Low Density lipoproteins having the same density but different peptide residues, but it is still necessary to establish that each polypeptide chain which has been found actually is part of a specific, and different, lipoprotein.

It has also been shown (18, 156) that the isotopically-tagged protein moieties of the High and Low Density lipoproteins do not equilibrate *in vitro* or *in vivo* and have different turnover rates (18, 156, 352). On the basis of these data and the N-terminal analyses it has been concluded that these two major groups of lipoproteins are probably separate in function and origin. This point is discussed further below.

It was proposed years ago (110, 242), on the basis of the charge observed on the chylomicron emulsion, that these particles contained a film of protein. Weld (364) suggested that some particles may have much less than others; and, considering the variation in surface area in particles differing in diameter from 0.1–1.5 μ, this would not be surprising. Bragdon (63), using an estimated thickness for protein monolayers (76), has calculated that a chylomicron of 0.5 μ diameter might be completely covered if its content were 1.5 per cent protein and this were all at the surface. The small amounts of protein present have been largely ignored until very recently. Middleton (256) reported that chylomicrons were flocculated by rabbit antibodies to human S_f 3–8 lipoprotein after the chylomicrons were first digested by steapsin. It is possible that his antigenic lipoprotein may have contained other than Low Density lipoproteins. Recently the chylomicron protein has been rather extensively studied by Rodbell (303), and Rodbell, Fredrickson and Ono (304–306). It is suggested by

these studies that the peptide residues found in exhaustively washed chylomicrons are specific, and not the result of random contamination of these particles with protein in the surrounding medium. Furthermore, they bear an interesting relation to other lipoprotein proteins. The delipidated chylomicron proteins from both dog and human lymph dissolve completely and may be subjected to electrophoresis in concentrated urea solutions. At least two, and usually three separate peaks are obtained. One of these contains N-terminal aspartic acid; following enzymatic digestion and chromatography after a method of Katz, Dreyer and Anfinsen (206), it yields small peptides identical to those obtained after similar treatment of the major High Density lipoproteins in that particular species. In human chylomicrons, the remaining proteins contain predominantly N-terminal serine; in the dog, one of the other chylomicron proteins has been determined to contain N-terminal glutamic acid. Protein labeling obtained upon *in vitro* incubation of dog intestinal mucosal cells with chylomicrons and labeled amino acids suggests that one or both of the principal polypeptides in chylomicrons are made in the gut (306). On feeding fat and isotopically labeled amino acids, the radioactivity is rapidly incorporated into the chylomicron protein (63, 306) and the specific activities in the High Density lipoproteins of the chyle and in the 'High Density-like' protein in the chylomicrons are identical (306), suggesting that these peptide residues, at least, are in rapid equilibrium. Injected into a recipient, the labeled protein of the chylomicrons also rapidly equilibrates with or transfers to the High Density lipoproteins of the plasma. It cannot be determined from these studies whether proteins identical to those in the High Density lipoproteins, or actually High Density lipoprotein molecules themselves, are found within or on the surface of the chylomicrons. It has been demonstrated that the cholesterol (136) and phospholipid (189, 244) of chylomicrons also rapidly transfer or exchange with that in other lipoproteins in the surrounding medium. Such transfer supports the theory that the intact High Density lipoprotein may be on the surface of the chylomicrons. Swank and Fellman (344) have recently reported that delipidation of washed dog chylomicrons leaves a protein with paper electrophoretic properties of an α_1-globulin which is consistent with the above findings. The apparent affinity of the High Density lipoproteins for these particles suggests they may serve as stabilizer as well as activator for lipoprotein lipase catalyzed hydrolysis (216). The possible function or relationship of the other chylomicron proteins described by Rodbell and Fredrickson (304, 305) must still be established. This is particularly pertinent to the problems of possible 'conversion' of chylomicrons to other lipoproteins.

A summary of current knowledge concerning lipoprotein structure and relationships is schematically presented in figure 1. No attempt has been made to conform to scale in particle size. It is desired to emphasize that at least three major groups of lipoproteins exist (in heavy contrast, fig. 1) so far as can be determined from the character of their peptide residues based on the available N-terminal amino acid analyses. The finding of lipoproteins, which apparently have polypeptide chains containing N-serine and N-threonine residues, predominantly in Low Density classes of high flotation rate up to and including the chylomicrons (303) warrants the inclusion of a fourth possible group or species of lipoproteins (in phantom contrast, fig. 1) which may actually be found in smaller quanties throughout the entire density spectrum. Further discussion concerning this lipoprotein grouping and lipoprotein 'interconversion' is presented below.

It should be mentioned that small amounts of lipid other than unesterified fatty acid may be associated with the protein residue sedimented in the ultracentrifuge at

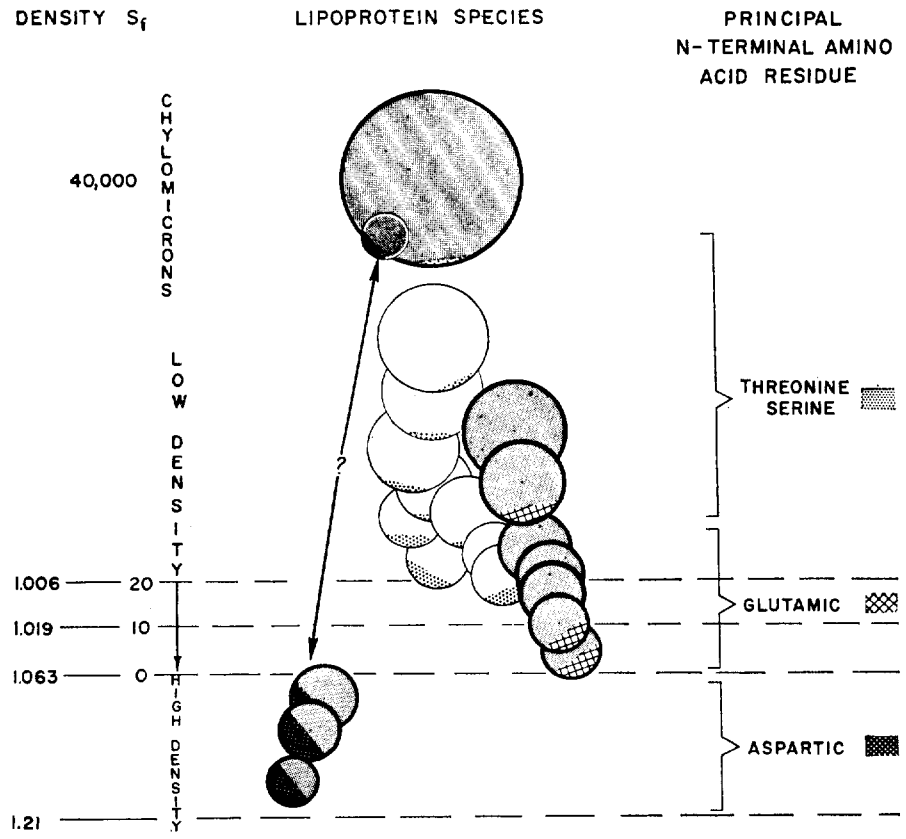

FIG. 1. Schematic conception of human plasma lipoprotein groupings according to present information. Polypeptide portions of the molecules are represented by the cross-hatching or stippling shown at the extreme right of the figure for the predominant N-terminal amino acid residue(s) present. No attempt has been made to conform to scale.

a density greater than 1.21 (146, 190, 195, 236). Lindgren *et al.* (236) suggest that there may be lipoproteins in the density range of 1.20–1.30. However, others have felt that this material represents largely non-lipid phosphorus (190) and no esterified fatty acids have definitely been demonstrated associated with proteins at these densities.

Stability of Lipoproteins. The general nature of the chemical binding and of the metabolic relationship of lipid to protein in the lipoproteins are problems germane to the study of fatty acid transport, but little specific information is available. Dervichian (97), McFarlane (245), Oncley *et al.* (273) and others have discussed possible types of bonding of neutral lipid and phospholipid to the lipoprotein protein. Since the soluble lipoproteins have chemical and physical properties of proteins rather than lipids, it has been assumed the peptide residues are on the surface of the molecule, where their charge will be most available (245, 273). Calculations based on protein content, estimated size of the protein molecules and thickness of protein films suggest that too little protein exists to cover the entire surface of soluble lipoproteins or chylomicrons (63, 245, 273). Hence, phospholipid may also promote surface stability,

since it contains a charged group. Chylomicrons lose their stability when exposed to the action of *Cl. welchii* lecithinase (111, 130, 297). Evidence that per-oxidation of lipid may produce instability of Low Density lipoprotein has been presented by Ray, Davisson and Crespi (291). Water is an important part of the lipoprotein molecule, the hydration of Low Density lipoprotein having been estimated to be about 0.6 gram of water per gram of protein (273), and water may facilitate the lipid-protein bonding (273). Removal of water produces irreversible changes in Low Density lipoprotein. In studying the removal of lipids from the lipoproteins, Avigan (17) found that practically all of the cholesterol in the $1.019 < \rho < 1.063$ lipoproteins could be removed without producing any apparent change in the protein residue. The phospholipids, however, could not be removed without denaturing the protein. On the other hand, the cholesterol in the High Density lipoproteins could not be removed without denaturing the protein, while the phospholipid could be more easily removed. Specific lipid molecules in the lipoproteins appear to leave and enter the lipoprotein macromolecule with relative ease under physiological conditions. Cholesterol has been shown to transfer readily from plasma to red cells (179), and from chylomicrons to High Density lipoproteins both *in vitro* and *in vivo* (136), and between High and Low Density lipoproteins (136). Free cholesterol appears to transfer or exchange much more readily than esterified cholesterol (136). Phospholipids also readily exchange between lipoproteins (108, 109, 189, 220, 244), and Lindgren has mentioned (236) observing the apparent transfer *in vitro* of labeled triglycerides between various Low Density classes. The triglycerides are readily available for hydrolysis by lipoprotein lipase (see below). James *et al.* (200) have recently shown that triglyceride and phospholipid synthesized in the red cell are taken up by lipoproteins upon *in vitro* incubation. Interestingly, the triglycerides transfer preferentially to the High Density lipoprotein, and the phospholipids to the Low Density molecules.

Chemical Composition of Plasma Fatty Acid Esters. Although the esterified fatty acids in plasma are similar in chain length to the plasma unesterified fatty acids, certain differences in the relative amounts of specific fatty acids in the different esters are present, and more may be revealed as analytical techniques continue to improve. Kelsey and Longenecker in 1941 (208) analyzed separately the fatty acids in cholesterol esters and triglycerides in beef plasma and found that 85% of cholesterol ester fatty acids were unsaturated, the largest component being linoleic, followed by linolenic and oleic. They found triglyceride fatty acids to be 56% saturated, being composed primarily of palmitic, stearic and oleic acids; about 18% linoleic, and 1% arachidonic were also present. The presence of polyunsaturated acids in large amounts in human plasma was demonstrated early by iodine number determinations (46) and later by spectrophotometric analyses combined with alkali isomerization (119). Using more specific techniques, Lipsky *et al.* (239) have recently reported that linoleic and oleic, with lesser amounts of palmitic and stearic, make up the major esterified fatty acids in plasma in man. Thus, the total esterified acids tend to be more unsaturated than the UFA (103). It has also been known for many years that the fatty acid composition of the several esters is also different in human blood (46). Lipsky *et al.* (239) found 86% linoleic and oleic acids in cholesterol esters, 65% in the phospholipids and 62% in the triglycerides. James and co-workers (199, 200) have chromatographed human plasma phospholipid and neutral fat (cholesterol esters plus triglyceride) fatty acids. They found acids ranging from saturated C_{10} acids to polyunsaturated C_{20} acids in both these fractions. Oleic acid predominated in both.

The bulk of human plasma phospholipid is known to be lecithin (177, 348), but 10–20% may be sphingolipids. The latter are known to contain unusually long-chain acids (84) which may have a function different from that of the C_{16-18} acids predominating in lecithin. There is also evidence that naturally occurring phospholipids contain unsaturated acids in the α position and saturated acids in the β position (181). Much more information is needed to understand fully the meaning of these positional differences in relation to fatty acid transport.

Some information is also available on possible differences in chemical composition within the various types of lipid esters found in different lipoproteins. Steele and Kayden (336) have reported that the proportion of sphingomyelin to lecithin was higher in Low Density than in High Density lipoproteins. James *et al.* (200) found some differences in phospholipid fatty acid composition, more long-chain, highly unsaturated fatty acids appearing in those of the High Density lipoproteins. Gillies, Lindgren and Cason (152) saponified the lipids present in isolated S_f 0–20 and 20–400 lipoproteins and determined the fatty acids by chromatography. They reported that the S_f 20–400 classes contained almost no polyunsaturated acids and about 20 per cent palmitoleic. The S_f 0–20 lipoproteins contained 4% tetraenoic and 18% linoleic, but almost no palmitoleic. They attributed the differences partly to the higher content of cholesterol esters in the S_f 0–20 class. The possibility that certain unesterified fatty acids might be preferentially bound to specific lipoproteins has previously been mentioned. Some preliminary data on the changes in fatty acid composition of the plasma esters with differences in ingested fat have been reported by Ahrens and co-workers (4). This promising area of investigation is just being opened and many further reports employing pure lipid fractions and more complete lipoprotein fractionations are awaited.

Turnover and Metabolism of Unesterified Fatty Acids

The physiologic significance of plasma unesterified fatty acid remained unsuspected for many years after the first discovery of its existence. In 1956 Dole (99) and Gordon and Cherkes (170) reported studies indicating that the fluctuations of the plasma level of UFA were related to the nutritional state of the subject. The latter authors formulated the hypothesis that UFA serves during fasting as a transport form of fatty acid, being added to the blood by peripheral adipose tissue, and removed as blood perfuses viscera. Since that time, reports have been published from a number of laboratories which appear to make possible a reasonable over-all picture of the physiologic behavior of UFA, though many significant details remain to be filled in.

Origin of Plasma Unesterified Fatty Acids. The unesterified fatty acids of blood plasma originate from one of three principal sources; the relative contribution of each is not constant, but varies with the nutritional state of the organism. The role of the gastrointestinal tract is relatively minor in most animals, and is specialized in that it contributes only short-chain fatty acids as UFA to the blood. This function is extraordinarily highly developed in the ruminants, which convert the majority of their intake of carbohydrates (including cellulose) to volatile, short-chain fatty acids by means of bacterial fermentation within the rumen (113). These fatty acids are absorbed and serve as a major energy source for the animal. Some of these fatty acids appear to be converted to other compounds by the epithelium of the rumen itself (280); others are absorbed unchanged (15, 16). It is reasonable to suppose that these short-chain fatty acids, which differ markedly from the higher fatty acids with

respect to their solubility and interaction with proteins, are not handled in the same way as the higher fatty acids in the UFA fraction. Their further fate is the subject of active investigation at this time; it must be remembered that the experiments on the fate of long-chain UFA, to be described below, do not apply to these short-chain fatty acids. A more complete account of the physiology of the rumen is beyond the scope of this presentation; the reader is referred to recent papers (20, 98, 114, 197, 327) for a more detailed account. Non-ruminants receive small amounts of preformed short-chain fatty acids in their food. Borgström (55) has demonstrated that the bulk of orally administered decanoic acid fed to the rat is absorbed into the portal vein blood as UFA. In a companion study, Blomstrand (39) demonstrated that the small fraction absorbed by the lymphatic route is primarily in the form of triglyceride. The presence of unesterified fatty acid in chyle, especially during fat absorption, has been reported several times (58, 139, 333, 368). Although there is some disagreement with regard to the actual quantity of UFA per liter of chyle, the values reported are such that the contribution of incoming chyle UFA to total plasma UFA turnover would be very small. Other investigators have noted that a variety of fed short-chain fatty acids fail to appear in lymph lipids both in man (127) and in rats (43). It is reasonable to presume that all the short-chain fatty acids are handled in the same way as decanoic. It would appear from recent studies by Samson and collaborators (311, 366) that rapid absorption of relatively large quantities of short-chain UFA has a detrimental effect on the central nervous system, at least in non-ruminants.

A second possible source of circulating UFA is the hydrolysis of plasma triglyceride. The production of UFA by the *in vitro* action of lipoprotein lipase (clearing factor) on the triglycerides of chylomicrons, lipoproteins and artificial fat emulsions has been shown repeatedly (56, 215, 298, 329). The same process is believed to occur *in vivo* following the parenteral administration of heparin (157, 185, 334). The nature of lipoprotein lipase and the evidence pertaining to its possible participation in intravascular UFA production is discussed in detail below. Whether or not hydrolysis occurs in the plasma *in vivo*, rises in plasma UFA levels have been reported during fat absorption (174, 300, 334), and after injection of artificial fat emulsions (174, 257) in the absence of exogenous heparin.

The third, and presumably most significant source of UFA in any quantitative sense is adipose tissue. The studies of Gordon (167) on intact, unanesthetized human subjects have shown by the measurement of arteriovenous differences that the source of UFA during fasting is a peripheral tissue. The most strikingly negative A-V difference was encountered in the greater saphenous vein, the content of which is primarily blood from subcutaneous areas rich in adipose tissue. The observation of Wadström (358) that the proportion of lower glycerides is increased in the depot fat of rabbits following the injection of epinephrine (which increases UFA mobilization) also indicates that adipose tissue produces UFA directly. The production of unesterified fatty acid from adipose tissue of rats incubated *in vitro* has been observed by several groups of investigators (171, 295). Net production of UFA from other tissue types, either *in vivo* or *in vitro*, has not been reported, so that, at least at this time, it must be considered that endogenous UFA (as opposed to UFA produced as a result of the degradation of dietary fat during alimentary lipemia) may originate solely from adipose tissue.

Removal of Unesterified Fatty Acids From Blood. Extraction of UFA from blood perfusing the human myocardium *in vivo* was demonstrated by Gordon and

Cherkes (170) and by Gordon (167). The technique used, that of measuring arteriovenous differences, resulted in data of the highest significance in the statistical sense, for the differences were many times the experimental error encountered in duplicate analysis. It is of interest that they found the fasting human heart to extract over one-fourth of the UFA presented to it, whereas after the administration of carbohydrate and glucose, the extraction ceased. These findings agree well with those reported earlier by Bing, Siegel, Ungar and Gilbert (34) who measured arteriovenous differences of total fatty acids of plasma. In the earlier studies, however, the differences to be measured were in the range of experimental error (as a much greater quantity of fatty acid was being determined), so that only the average of a series of determinations had statistical validity. The only other clear-cut example of extraction was that found by measuring A-V differences in the hepatic vein (170); these showed that the splanchnic area extracts UFA as avidly as does the myocardium. Skeletal muscle would appear to extract UFA, but the results presented by Gordon (167) are erratic, and reflect the presence of adipose tissue, contributing UFA, and muscle, extracting it, in the extremity from which the blood was obtained. Similar arteriovenous differences demonstrating the extraction of UFA in the tissues of experimental animals have been observed by Olson (271), and Spitzer and Roheim (335). The demonstration of these arteriovenous differences in man and animals establishes the transport function of UFA beyond any doubt.

Removal of UFA from the blood can also be shown by the use of isotopic fatty acids. If such a tracer is injected into the plasma and then recovered from another part of the body, there can be little doubt of its removal from the blood. There are, however, two complicating aspects to such a study. First, the fatty acid may not have been removed by the organ or tissue in which it is found, but rather removed elsewhere and transported in another form to the site of its final recovery. This problem can be minimized if the time between administration and recovery is short. Secondly, the presence of the tracer in tissue need not be the result of net removal of UFA from plasma to that tissue. A tissue containing a pool of fatty acids, fixed in size, that can exchange with plasma UFA, will become labeled after the administration of label intravenously, but may not be removing UFA from the blood passing through it.

Bragdon and Gordon (67) reported on the radioactivity of the tissues of rats given carbon-14 labeled UFA or chylomicra intravenously. Each study was performed in fasted as well as carbohydrate-fed rats. It is noteworthy that the UFA left the plasma for the tissue in either nutritional state. Virtually all tissues of the rat became labeled; only the brain, of the tissues examined, was slightly radioactive. Dole (101, 102) cites similar studies in his own laboratory, the details of which have not all been published. He observed that the tissue radioactivity was not all still present as unesterified acid, but rather that the label had been incorporated into triglycerides and phospholipids.

The first measurements of the rate of removal of UFA from the blood were reported by Havel and Fredrickson, who injected palmitate-C^{14} complexed to albumin intravenously into a dog and obtained a half-time of about 2 minutes for the earliest phase of removal (191). The rapid disappearance of UFA from the plasma of rabbits and dogs has been reported by Bierman, Schwartz and Dole (33) and Fredrickson, McCollester, Havel and Ono (136, 137), and of humans by Fredrickson and Gordon (133, 134) and Laurell (227). That UFA is removed from the blood, and rapidly, can also be deduced from the studies of Lossow and Chaikoff (241) and McCalla, Gates and Gordon (243) in which carbon-14, administered to rats as UFA, appeared in the

expired air as $C^{14}O_2$. The same phenomenon has been observed in dogs (136, 137) and in humans (133, 134).

Extraction of UFA from media by tissues incubated *in vitro* has been demonstrated by several laboratories. Some studies have utilized short-chain fatty acids in media of low protein content, but others have demonstrated similar effects where the substrate was albumin-bound long-chain UFA. Shapiro, Chowers and Rose (325) and Stern and Shapiro (337) have reported the removal of UFA from serum by adipose tissue; the same occurrence was noted in one experiment reported by Gordon and Cherkes (171). Masoro and Felts (253) have observed utilization of UFA by liver slices incubated *in vitro*, and Mead (127a) has noted similar utilization by ascites tumor cells. Consumption of UFA by skeletal muscle *in vitro* is mentioned by Fritz and co-workers (145), and the oxidation of short-chain UFA (octanoate) by rat diaphragm is reported by Neptune and collaborators (266).

The prominent uptake of UFA by adipose tissue *in vitro* (see above) might appear to contradict the evidence of Bragdon and Gordon (67), who find that C^{14}-labeled UFA administered intravenously to rats is taken up only slightly by adipose tissue. No contradiction need exist, however, when one considers that the result of the *in vivo* experiments depend on the competition between adipose and other tissues for the tracer. If adipose has a relatively poorer blood supply, or a smaller intracellular pool of exchangeable fatty acids, one would expect less activity in adipose than in other organs.

One can only speculate regarding the mechanism of the removal of UFA from plasma into tissues. That UFA is strongly bound to albumin has been proved (see above), and yet there is an enormous contrast between the rate of removal of UFA from the blood *in vivo* and the rate at which albumin is removed into the tissue spaces (29). There can be no escape from the conclusion that the UFA is separated from its carrier protein as it moves into the tissue. The UFA-binding sites which Goodman (164) has observed on the stromata of red cells may be the prototype of similar receptors located on all cells which have the capacity to remove UFA by binding it with an affinity that is at least similar to that of albumin. In the removal of UFA from blood by such a tissue as myocardium, the first step would be expected to be removal from circulating albumin to vascular endothelium, and a second step, which may be facilitated by extravascular albumin, would be the transfer of UFA from endothelium to the parenchymal cells. The details of the mechanisms responsible for the speed and efficiency of this transfer will make a challenging field for future study.

Transport Function of Unesterified Fatty Acids. The evidence that established the transport function of UFA leads naturally to the problem of the quantitative evaluation of this process in relation to the over-all metabolic needs of the organism. If UFA serves during fasting as an important substrate for cellular oxidation and energy production, the quantity of fatty acid moved by this route should represent a large fraction of the total energy production, as measured in calories, or as evidenced by oxygen consumption or carbon dioxide evolution. It is possible to carry out such a comparison for individual tissues when suitable arteriovenous differences have been measured. This approach, however, is limited to tissues whose anatomy and vascularization is satisfactory, and, of course, can never apply to the whole organism. Only in the case of the myocardium of man and dog have enough measurements been made to give assurance that UFA extraction is great enough to provide a major share of the chemical energy supplied to the organ. By calculating oxygen extraction ratios (defined by Bing *et al.* (34) as the percentage of the observed oxygen extraction that

might be accounted for by the complete oxidation of the substrate whose extraction is being measured simultaneously), Gordon (167) using human subjects and Olson (271) using dogs, have arrived at the conclusion that UFA is a major energy source for heart muscle in the fasting state. Gordon (167) demonstrated in one case that the provision of carbohydrate calories was followed by rapid cessation of extraction of UFA by myocardium. Data on the extraction of UFA by the splanchnic bed have also been recorded (167); but in this case, the observed A-V differences cannot be applied to a specific tissue, since hepatic vein blood will represent drainage from liver, GI tract, and mesenteric adipose tissue, mixed in an unpredictable fashion. A second objection to the use of oxygen extraction ratios is that such studies, alone, cannot prove that the substance being extracted is actually being used as a source of energy. If it is undergoing some other fate, comparison with oxygen utilization is not pertinent. In the case of UFA extraction by myocardium, however, no reasonable alternative route of disposition has been suggested. In the case of liver, utilization of UFA for the synthesis of more complex lipids is a possibility, as is conversion of UFA to ketonic acids. The latter process has indeed been shown to occur in uncontrolled diabetes (168).

The other approach to the question of the quantitative evaluation of plasma UFA as the source of energy production during fasting is the study of the kinetics of the conversion of isotopic UFA to carbon dioxide. From such data one could hope to estimate the relationship of production of CO_2 from UFA to total CO_2 production. Studies of this type have been undertaken by Fredrickson, McCollester and Ono (136, 137) in dogs, and by Fredrickson and Gordon (133, 134) in human subjects. In each study, C^{14}-carboxyl-labeled UFA was injected intravenously into the subject, and serial samples of blood and expired air analyzed for radioactivity. The studies on dogs were carried out exclusively with palmitic acid, but the human subjects received three different acids—oleic, linoleic and palmitic. Each of these acids was found to behave similarly with regard to the rapidity of the initial phase of their removal from plasma. This component of the curve of plasma radioactivity against time had a half-time in the range of 1–3 minutes. From this half-time, the plasma concentration of UFA, and the plasma volume, a figure could be calculated for the rate of turnover of the intravascular UFA pool. The results in the human agree well with those of Laurell (227), and indicate a plasma turnover varying from 0.1 to 2.0 mEq UFA per minute. The plasma UFA turnover was reduced after administration of carbohydrate, but not abolished, as was the case for A-V differences of UFA (167). Indeed, a decrease in the half-time for removal of label partly compensated for the decrease in plasma UFA concentration, and plasma UFA turnover fell less than might be anticipated (134). After an initial rapid phase of disappearance, plasma radioactivity dropped more slowly, and several slower exponential components could be discovered in the plots. This observation was taken to indicate recycling, or the reappearance in the plasma of labeled UFA molecules that had once been cleared. In addition, label appeared in other plasma lipid fractions (134). Recycling was especially prominent with linoleic acid. Since the plasma UFA turnover in fasting humans is approximately equivalent to total CO_2 production, or is roughly equivalent in terms of caloric equivalents to the daily energy requirement (133, 134, 227), it would be necessary that recycling be negligible in order for oxidation of plasma UFA to account for 100 per cent of the CO_2 output. Actually, the total amount of C^{14} moving through the plasma compartment as UFA exceeded by several-fold the total amount expired as CO_2, leaving the inevitable conclusion that all UFA removed from plasma is not

directly oxidized. Rough estimates of the contribution of UFA carbon to total CO_2 production (134) indicate that this substrate cannot supply more than 50 per cent of the total demand for energy in the human subject fasted 16 hours. A more detailed mathematical interpretation of these turnover curves, which may fix this figure with greater precision, is now in progress (338).

There are several factors that limit the reliability of studies of this type. Low levels of radioactivity obtained in the later portions of the decay curves make errors inevitable in these slopes, yet they are of considerable importance in the interpretation of the data. This is particularly true in healthy human subjects, in whom the most conservative doses of radioactivity must be administered. Secondly, it is necessary to assume that the appearance in CO_2 of the carboxyl carbon from a UFA molecule signifies complete oxidation of the fatty acid. That this is true in rats is strongly supported by the findings of Weinman *et al.* (361), but a corresponding study has not been performed in man. Unfortunately, the randomly-labeled fatty acids are much less readily available in pure form and high specific activity than are the carboxyl-labeled ones. Finally, it is necessary to assume that the plasma UFA fraction all behaves in the same fashion as the particular labeled acid injected. This assumption cannot be in great error, since oleic and palmitic acids, which together comprise over half of the circulating UFA (100, 103), behave very much alike with regard to kinetic studies. However, differences already observed in the plasma turnover of linoleic (134) will make it necessary to determine the identity of the acids in which the injected radioactivity may be found after its injection.

There is one other possible approach to the quantitative evaluation of plasma UFA as a transport form of fatty acid which has not yet been exploited. In the fasting state, where adipose tissue is the only important source of circulating UFA, it might be possible to estimate simultaneously the total blood flow through adipose tissue and the average A-V difference of UFA across the tissue. The practical difficulties that would be encountered in such an experiment would be formidable, but a valid result should be satisfactory for unambiguous interpretation.

Regulation of Unesterified Fatty Acid Metabolism. The factors controlling and affecting the transport of fatty acids as UFA are the subject of active investigation at this time. The literature available at present includes a wide variety of observations, the majority of which are based on experiments in which one or another operation was found to affect the concentration of circulating UFA. In a lesser number of studies, experimental operations were shown to alter the rate of turnover or transport of the fatty acids.

The most clearly established relationship of this group is that between carbohydrate metabolism and the metabolism of UFA. Administration of carbohydrate to a previously fasting animal or human produces a sharp decrease in circulating UFA concentration (99, 170); the only exception to this rule is found in severe diabetes mellitus (31, 226), in which state it is presumed that utilization of the carbohydrate is defective. The same fall in UFA concentration is produced by tolbutamide (32), by injection of glucagon, which mobilizes endogenous glucose (31), and by injection of insulin (99), which accelerates the utilization of endogenous glucose. It is evident that two of the above-mentioned stimuli produce a rise in blood glucose concentration, and the other two cause a fall; the common factor is that all directly or indirectly increase the rate of utilization of glucose in peripheral tissues. (The evidence that this is true of tolbutamide is not definitive.) That the UFA response to acceleration of glucose utilization is due to a decrease in the rate of liberation of UFA from adipose

tissue seems established. Bierman, Schwartz and Dole (33) observed that, in the case of animals whose circulating UFA had been labeled by the prior injection of UFA-C^{14}, the administration of insulin caused a decrease in plasma UFA concentration accompanied by a rise in specific radioactivity. This result was interpreted to mean that insulin had decreased the rate of inflow of endogenous (unlabeled) fatty acids into the plasma, but had not accelerated the rate of removal by other tissues. A corresponding effect was observed when the radioactive acid was administered by constant infusion. Gordon (167), on the other hand, found in human subjects that the arteriovenous differences characteristic of active UFA transport were abolished by the administration of glucose and insulin together. Simultaneously, the circulating concentration decreased. These changes could occur together only if the primary event were a decrease in the rate of production of UFA in the periphery. Finally, Gordon and Cherkes (171) have shown that adipose tissue incubated *in vitro* will decrease its rate of UFA production under the influence of glucose and insulin.

An effect of carbohydrate on the rate of removal of UFA by the tissues that consume these fatty acids is suggested by the observations of Laurell (227) and of Fredrickson and Gordon (134), who agree that the fractional rate of removal of labeled UFA from plasma is increased after carbohydrate. The decrease of plasma concentration is such, however, that the rate of removal in milliequivalents per minute is actually considerably reduced, and these data do not conflict with those mentioned above. The primary role of the adipose tissue in the regulation of UFA levels during fasting and carbohydrate feeding seems established.

The feeding of amino acids to human volunteers was reported by Gordon (167) to cause a decrease in circulating UFA concentrations. The change was less striking than that produced by glucose, but had about the same duration. This effect can be attributed to the adipose tissue only on the basis of its analogy with the observed glucose effect. Ingestion of fat by experimental subjects, human or animal, produces less clear-cut effects. Grossman, Moeller and Palm (174) and Robinson *et al.* (300) report rises in UFA concentration in human subjects given fat, whereas Gordon and Cherkes (170) and Dole (99) do not find this effect reproducible. Grossman *et al.* (174) and Spitzer and Miller (334) have noted increases in UFA concentrations in animals given fat. It is probable that the variation in experimental results which has been noted in these experiments is not of great significance. The fasting subject does not alter his UFA transport processes in any fundamental way when exogenous fat is administered alone, but he may partially substitute the dietary fat for stored fat as a source of UFA (191). Simulating fat absorption by infusion of chylomicrons at a rate considered physiological, Fredrickson, McCollester and Ono (137) estimated that about 10 per cent of the plasma UFA might be coming from dietary fat under the conditions of their experiments in dogs. Whether or not a change in UFA concentration will result during fat absorption will probably depend on many factors, including rate of absorption, rate of transfer of triglyceride from the blood, rate of hydrolysis of triglyceride, rate of utilization of UFA, and responsiveness of adipose tissue to the exogenous supply of fat. When exogenous fat is administered abruptly and in large quantity parenterally as a fat emulsion or a triglyceride-rich lipemic plasma (81, 192), a clear-cut increase in circulating UFA concentration occurs.

A variety of hormonal agents, no one of which is free of effects on the metabolism of carbohydrates, have also been shown to affect the metabolism of UFA. Epinephrine causes an increase in UFA levels (99, 170), an effect which is presumably attributable to its effect on adipose tissue (171). Norepinephrine behaves similarly when tested on

adipose tissue (365). Growth hormone has been reported to increase UFA levels when injected into intact humans (289) and hypophysectomized rats (115). An effect of growth hormone on the lipolytic activity of isolated adipose tissue *in vitro* has been observed by White and Engel (365). Adrenocorticotrophic hormone also increases UFA production by adipose tissue *in vitro*, according to the latter authors, and this effect is not attributable to contamination with growth hormone.

Finally, and perhaps not of least importance for those interested in performing investigations in this field, is an effect observed by Gordon and Cardon (169), attributed to psychic stress. In this study, 13 human volunteers were told that an unpleasant procedure was to be performed. Within 5 minutes, their UFA levels rose appreciably above control values obtained just prior to the production of anxiety.

These many observations can be fitted together, at least tentatively, in terms of a concept of 'caloric homeostasis.' Since dissimilable substrate must be available for the production of energy at all times, an organism must, if it is to survive, have a means of mobilizing reserve supplies when exogenous foodstuffs are in short supply. On the other hand, efficiency demands that such mobilization be halted when exogenous food is obtained. This homeostasis is a function of the adipose tissue, which liberates UFA into the blood when no other source of energy is available. The means whereby the adipose tissue itself is controlled are not known as yet. It is not out of the question that this tissue serves as its own chemoreceptor, and releases UFA when the blood with which it is perfused is relatively poor in non-fatty acid substrates. The effect of glucose and insulin on isolated tissue is compatible with this hypothesis. The effects of the various hormones and of the central nervous system would suggest that these agents either alter the availability of non-fat substrates to the adipose tissue (or to its chemoreceptor if it be located elsewhere), or serve as direct stimuli synergistic with the stimulating effect of shortage of non-fat substrates.

Lipoprotein Lipase

The enzyme, lipoprotein lipase (215), which catalyzes hydrolysis of triglyceride in lipoproteins and specially prepared fat emulsions, is of considerable interest and probably of wide physiological importance in the field of fatty acid transport. It will be helpful to review briefly what is known about this enzyme before discussing the transport of fatty acids by glycerides. Lipoprotein lipase isolated from plasma, adipose tissue and myocardium (215, 216, 218) appears to be identical to 'clearing factor' first observed after injection of heparin into lipemic animals (12, 180, 363). The literature pertaining to 'clearing factor' or lipoprotein lipase is extensive and has recently been reviewed by Bergström and Borgström (26) and by Robinson and French (299).

Following the observations *in vivo* and *in vitro* that post-heparin plasma contained a factor which could decrease visible lipemia in blood (12, 13, 143, 180, 363), it was demonstrated that such clearing was the result of hydrolysis of the triglycerides in the large, light-scattering, triglyceride-rich lipoproteins (215, 298, 329), with acceptance by the serum albumin of the fatty acids released by hydrolysis (172, 298). After the partial purification and determination of some of the properties of the 'clearing factor,' Korn (215) adopted the term lipoprotein lipase to identify the nature of the activity of this enzyme and to distinguish it from pancreatic lipase. A number of other studies defining the different properties of these two enzymes are summarized elsewhere (299). Lipoprotein lipase is inhibited by protamine and basic dyes (70, 72, 92, 269), by detergents (70, 301) and by heparinase (217). Beyond the fact that

heparin appears to form an essential part of the enzyme complex, nothing further is known about the manner in which heparin causes lipoprotein lipase activity to appear in the blood, presumably 'spilling out' from the tissues. This property of heparin is not unique and is shared by other heavy anions (188). Swank and Levy (345), measuring A-V differences by chylomicron counts after heparin injection, concluded that various tissues differed in the release of 'clearing factor,' and that the area served by the cerebral circulation contributed none. The observations reported by Swank (343, 346) of *in vitro* clumping of chylomicrons, especially in post-heparin plasma, may represent some action of heparin independent of its relationship to lipoprotein lipase, possibly identical to the aggregation of Low Density lipoproteins by dextran sulphate (276). Any relationship of this phenomenon to the normal removal of chylomicrons (see below) is doubtful. Lipoprotein lipase acts specifically upon the ester linkages in triglycerides (328), not attacking the cholesterol esters or phospholipids. Production of lower glycerides has been observed both *in vitro* and *in vivo* (82, 83). The enzyme is adsorbed tightly to the surface of chylomicrons or fat emulsions (215, 302) and it is possible that a lipoprotein, specifically High Density lipoprotein (216), may facilitate this bond and thus serve to 'activate' particles of triglyceride for enzymatic hydrolysis. Robinson and Harris (cited in 299) have reported activation of triglyceride emulsions by phosphatide alone. Hydrolysis is catalyzed in all lipoprotein substrates which contain sufficient triglyceride for adequate measurement (216, 236) and proceeds as long as there is adequate receptor for the product fatty acids. The changes in lipoproteins which result from hydrolysis of the triglycerides are summarized in a following section.

It has not been conclusively proved that lipoprotein lipase has a specific role under physiological conditions (although this is very likely) and it has particularly not been satisfactorily demonstrated that plasma lipoprotein lipase activity is important. Spontaneous activity in the absence of exogenous heparin has been observed in man (116), and in certain strains of rats after fat feeding (201), such activity being increased by removal of the liver from the circulation (202, 302). By extrapolating *in vitro* measurements of activity to the intact man, it has been estimated (149, 187) that plasma lipoprotein lipase activity alone might account for only a small percentage of the clearing of ingested fat entering the blood during fat absorption. The removal of chylomicrons in animals does not appear to depend upon plasma lipoprotein lipase activity (191), and may occur in isolated tissues (263), or the intact animal (138), even though the hydrolytic mechanism be impaired by protamine or other inhibitors. The small changes in composition of plasma unesterified fatty acids observed with fat feeding in man (103) and the estimated contribution of chylomicron triglyceride to the unesterified fatty acids during chylomicron removal in the dog (137) also do not support the concept of wholesale splitting of chylomicron triglycerides within the blood stream in alimentary lipemia. The evidence for lipoprotein transformations as discussed below is compatible with, but does not prove, a role of plasma lipase activity in this process. The majority of evidence seems to favor the intervention of hydrolytic activity, within or on the surface of tissue cells, as distinguished from intravascular hydrolysis alone, as accounting for most of the triglyceride hydrolysis associated with chylomicron clearing and other lipoprotein transformations. It must be borne in mind, however, that differences in species in this regard may exist. There is, of course, a good possibility that other lipases, particularly in adipose tissue (288, 294) may have important physiological roles in fat transport and metabolism. The lipases found in mammalian tissues have recently

been reviewed by Overbeek (277). No attempt will be made here to cover the many reports of clinical investigations which have been made into the response of patients to heparin as measured by lipoprotein lipase activity. It may be mentioned, however, that Klein and Lever (213) have reported finding that serum of patients with idiopathic hyperlipemia contains a factor which inhibits *in vitro* lipoprotein lipase activity. Havel (186) has studied one family containing three hyperlipemic members who showed no lipoprotein lipase activity in plasma after heparin. Their plasma contained no demonstrable inhibitor for normal lipoprotein lipase activity.

Transport of Exogenous Fatty Acids as Glycerides

Even before it was definitely established that ingestion of fat led to transient rises in blood lipids, it was apparent from the now classical observations of Munk and Rosenstein (265) that lymphatic pathways were of great importance in the transport of exogenous fatty acids. As Gage and Fish point out in their review (148), investigators in the seventeenth and eighteenth centuries suspected that dietary fat was responsible for a milky appearance in chyle, and knew that this material found its way from intestinal lacteals to the blood primarily by way of the thoracic duct. Several experiments in this country, employing thoracic duct fistula animals, established that fatty acids fed either free or as glycerides were absorbed into the chyle (49, 107, 140, 240). Once isotopically-labeled fatty acids became available and were added to this technique, knowledge about fatty acid transport began to accumulate at a tremendous rate, which still shows little sign of slackening.

In 1950, Bloom *et al.* (41) fed palmitic acid-C^{14} to rats as either tripalmitin or free fatty acid and recovered 70–92 per cent of the absorbed acid in either intestinal or thoracic duct lymph. At about the same time, Bergström *et al.* (27) fed stearic acid-C^{14} to cats, and obtained most of the radioactivity in chyle as glyceride. Borgström (50) found 85 per cent of absorbed palmitic acid in thoracic duct lymph, and 97 per cent of this as glyceride. Repeated studies of this type have subsequently established that longer chain ($> C_{10}$) fatty acids, either saturated (27, 38, 41–43, 50, 52, 53, 211) or unsaturated, such as oleic (25, 38) and linoleic (38), are preferentially carried as glyceride in the thoracic duct lymph. Less than half of absorbed decanoic acid-C^{14} appears in the lymph (39, 55, 211), although that portion which is present is 99 per cent as glyceride (39); the larger part leaves the intestine via the portal circulation, here largely as unesterified fatty acid (55). As is discussed below, appreciable amounts of some acids, such as stearic, appear in the lymph also as phospholipid. Only small amounts of these fed acids appear in the lymph as cholesterol ester (27, 52) or as unesterified fatty acid (58). Carlson and Wadström (82, 83) have reported finding that about 10 per cent of the glycerides in both human chyle and plasma were di- and monoglycerides. This data, based on silicic acid separations according to Borgström (51), show no difference in the proportion of lower glycerides in blood obtained during fasting and during alimentary lipemia, in the absence of exogenous heparin. Mead and Fillerup (254) have fed the methyl esters of labeled stearate, oleate and linoleate to rats and studied the distribution of radioactivity in silicic acid fractions of the plasma lipids. They found that most of the radioactivity of the first two acids remained in the glycerides for several hours, while a much higher proportion of the linoleic acid was present in phospholipids. About 20 per cent of the labeled fatty acids appeared to be in di- and monoglycerides, although this does not imply that this proportion of the fatty acids found in lower glycerides in plasma also prevailed in lymph. Further experiments are needed on the implications of lower glycer-

ides in the blood, with particular reference to possible species differences. In the subsequent discussion we will not attempt to distinguish between triglycerides and possible lower glycerides which may be present.

Origin of Chylomicrons. During the absorption of a typical long-chain fatty acid, such as palmitic acid, the triglycerides emerge into the lacteals in stable emulsions in the form of chylomicrons. Since particles of approximately 0.1 μ and more scatter light, the chyle becomes lactescent. No extensive study of the various Low Density lipoprotein classes in lymph during fat absorption appears to have been published, but it is apparent that most of the triglyceride present at this time is in the form of chylomicrons (9, 10, 57, 78, 351). It is not known whether any significant changes in lymph lipoproteins may occur as absorption proceeds, although, during the major part of fat absorption in the rat, Borgström and Laurell (57) noted that the ratio of total lipid to light-scattering (a rough index of particle size) remained relatively constant. Earlier workers like Gage and Fish (148) and Ludlum *et al.* (242) had reported seeing fluctuation in mean particle size during absorption.

The chemical nature of the protein adherent to the chylomicrons, and the rather tentative evidence (303–306) that some of this protein may be synthesized in the gut, have been summarized above. The presence of a peptide chain identical to that found in the High Density lipoproteins (304, 305), plus small, but rather constant amounts of cholesterol and phospholipid, indicates that probably intact High Density lipoprotein is present to stabilize the emulsions as they are formed. There is evidence that other polypeptides (304, 305) and 'extra' cholesterol and phospholipid are also present (234). Bragdon (63) has found it possible to increase the amount of cholesterol in lymph chylomicrons by feeding high cholesterol diets, but feeding excess lecithin does not alter the phospholipid content. The latter may be due to the hydrolysis accompanying absorption of most fed phospholipid (40, 44). Since the diameter of chylomicrons present during absorption varies several-fold (148), differences in surface area will consequently be much greater and it is possible that the amount of chylomicron surface covered by protein or phosphatide will differ greatly among the particles. The nature of the processes by which the intestinal mucosal cell places the longer-chain fatty acids in remarkably stable triglyceride emulsions and shunts those of lesser chain length directly into the portal circulation is presently largely a matter of speculation. A recent review of investigations concerning absorption has already been cited (26).

Metabolism of Chylomicrons. Once chylomicrons enter the blood, they are normally rapidly removed, although a transient rise in total blood fatty acids is a normal accompaniment of moderate fat ingestion (249). The process of chylomicron removal is still only partially understood and should not be considered necessarily analogous to the 'clearing reaction' following heparin administration, which was discussed above. Up to recent years this problem has been explored either indirectly by chylomicron counts in the blood, or through injection of fat emulsions, which have not always represented physiological substrates. Bloor was among the first American workers to employ the latter technique and he reviewed (45) earlier European work, in which it was found that intravenously injected colloidal particles of oil, or of egg yolk, rapidly disappeared from the blood. Subsequent reports of studies with fat emulsions have reviewed the later work with this technique (264, 356). Since each of the preparations used may differ in particle size, fat content and nature of stabilizer, and because certain of the results show tissue distribution and rates of oxidation which differ from recent studies employing actual lymph chylomicrons, it is difficult

to interpret all of these studies in terms of normal chylomicron metabolism. However, good evidence that some fat emulsions may represent substrate readily available for caloric purposes (23, 150, 160, 229, 264, 322, 356) and have behavior similar to ingested fat (353) has been presented in specific instances. Artificial triglyceride emulsions may be removed rapidly, and at rates dependent upon initial loading or the total amount of fat infused (354, 357). The sites of their removal have not been well studied, in the light of what is now known about the importance of unesterified fatty acid transport in chylomicron metabolism, but hepatectomy has been shown to delay removal and oxidation of the fatty acid moieties (151, 160).

The metabolism of chylomicrons containing isotopically-labeled fats or protein, and obtained from the lymph of donor animals fed appropriate precursors, has been a major interest of this laboratory. Havel and Fredrickson (191) injected intravenously chylomicrons in which the triglycerides contained palmitate-1-C^{14}, and observed that over 90 per cent of the injected load disappeared from the blood at an exponential rate in anesthetized dogs. Comparison of the early rates of disappearance with those obtained upon injection of smaller amounts of triglyceride (136) indicated that the greater the load, the slower the fractional rate of disappearance. This has also been observed in rats by French and Morris (141, 142) who found that an initial load also delayed the disappearance of subsequently injected chylomicrons. Havel and Fredrickson (191) also separated serial plasma samples from the recipient dogs into lipoprotein fractions by ultracentrifugation, and observed very little radioactivity in the lipoproteins corresponding to about $S_f < 400$ during the removal of chylomicrons. Many hours later the labeled triglyceride fatty acids emerged in Low and High Density lipoproteins, largely in phospholipid. During the removal of the labeled chylomicron triglycerides, it was observed that the labeled fatty acids rapidly appeared as unesterified fatty acid (191). In these animals no change in total unesterified fatty acid concentration was observed, despite the infusion of over 300 milligrams per kilogram total lipid as chylomicrons. Fredrickson, McCollester and Ono (137) have compared the flux of radioactivity in blood unesterified fatty acids and in the expired CO_2 after administration of labeled palmitate as the albumin-bound acid and as chylomicron triglyceride fatty acid to dogs. While the total carboxyl carbon excreted as CO_2 was about the same in both cases, much less radioactivity traversed the plasma as unesterified fatty acid after chylomicrons were injected, and it was concluded that retransport of triglyceride fatty acids in the unesterified form is not a necessary step for their oxidation. From further mathematical analysis of these data, it can be concluded that over half of the chylomicron triglyceride fatty acids were directly oxidized (338). The rapid oxidation of the fatty acids in chylomicron triglyceride also has been demonstrated in the rat by Morris (259) and by Bragdon (62). In the dog, the flux and apparent dilution of the labeled carboxyl group in the CO_2 (136, 137) during the oxidation of injected palmitic acid is very similar when the acid is injected as triglyceride or in the unesterified form, except for an initial lag period following injection of the esters, leading to the speculation (137) that much of the chylomicron triglyceride may be removed directly by the same tissue sites oxidizing plasma unesterified fatty acids. Bragdon and Gordon (67) have observed that labeled palmitate, injected intravenously into fasting rats in either substrate form, has a similar tissue distribution, largely in liver and skeletal muscle. Adipose tissue, however, became labeled only when the palmitate was injected as chylomicra. French, Morris and Robinson (142) have noted that hepatectomy caused a delay in removal of chylomicrons, and Morris and French (263) have found the isolated, perfused

liver capable of removing and oxidizing chylomicron triglycerides. Gage and Fish fed Sudan-labeled fat (148) with carbohydrate and found that it appeared rather quickly in the adipose tissue and that certain depots appeared to have a higher turnover or greater affinity for the dye.

Although it seems well established that hydrolysis is an almost immediate accompaniment to disappearance of chylomicrons, the evidence obtained from animal experiments is against the necessity of intravascular hydrolysis operating in the normal removal of chylomicrons. In addition to the observations of Havel and Fredrickson (191), Bergström *et al.* (28) had previously observed a lack of equilibration between incoming particulate (chylomicron) triglyceride and the remaining plasma triglyceride during fat absorption in rats. In the isolated liver experiments of Morris and French (263), Triton, which is a potent inhibitor of lipoprotein lipase (70, 301) did not retard the uptake of chylomicrons, although the oxidation of the fatty acids was delayed. Fredrickson, McCollester and Ono (138) have found that in the intact dog given protamine, small loads (60 milligrams total lipid per kilogram) of chylomicrons were removed at a normal rate, although the reappearance of the fatty acids in the blood in the unesterified form and their oxidation were markedly slowed. Since protamine and Triton have been shown to delay the removal of larger amounts of chylomicrons (68, 141), it is possible that uptake of these particles by the liver, for example, involves sites which need to be quickly cleared through hydrolysis and redistribution of lipid in order to renew their capacity for removal. This is further suggested by the retardation of clearing of chylomicrons following a previous load and the dependence of the fractional rate on the plasma concentrations (141, 142).

Assuming hydrolysis is not essential for departure of chylomicron triglyceride, the actual process of transfer across the capillary wall is not understood. Because of early results with fat emulsions suggesting removal by the reticuloendothelial system (246), and from consideration of the size of the chylomicrons, attention has been focussed on the R-E cells. In the experiments of Friedman, Byers and Rosenman (144), 'blockage' of the reticuloendothelial cells by colloidal substances appeared to delay chylomicron removal, although Waddell *et al.* (355) have found similar techniques to have no effect on removal of artificial fat emulsions. During removal of perfused chylomicrons, the liver has been observed to contain more discrete fat droplets in the parenchymal cells than in the Kupffer cells (263). The careful studies of Morris and Courtice (93, 260–262) on lipid exchange between plasma and lymph have established that chylomicrons can move, apparently intact, from plasma to hepatic and thoracic duct lymph. In fact, once free in the extravascular space, chylomicrons can gain access to the lymphatic system anywhere in the body (93). Using the electron microscope, Fawcett (125) has reported the presence of large spaces (up to 1 μ in diameter) in the sinusoidal endothelium of the liver. These conceivably could accommodate the direct passage of chylomicrons from sinusoids into the hepatic lymph. Considering the speculations of Renkin (293) that lipid-soluble substances may find it much easier to diffuse through the capillary endothelial cell wall than through the water-filled capillary pore, more attention must also be given to the possibility that chylomicrons may take this route from the capillary into extracellular spaces and then into tissue cells. Indeed, the retardation of chylomicron removal by charged particles such as protamine and certain detergents may not be related only to their inhibition of an enzyme such as lipoprotein lipase, but also to their surface effects at cell walls. In this regard, Tween 80 has been shown to raise the plasma-lymph gradient for lipid (261). At present chylomicron removal must be considered possible by a number of mech-

anisms, including phagocytosis of larger particles and direct diffusion through capillary walls or 'pores' into tissue cells. McCandless and Zilversmit (244) have suggested that chylomicron phospholipid leaves the plasma more slowly than the triglyceride; Havel and Clarke (189) have interpreted similar experiments as indicating that labeled chylomicron phospholipid and triglyceride probably leave the plasma at the same rate except for some net accumulation of phospholipid in High Density lipoproteins during removal. It is also possible that some of the protein 'membrane' may be left at the capillary wall during chylomicron removal, since in the experiments of Rodbell, Fredrickson and Ono (306) labeled chylomicron protein remained in the blood long after the chylomicron triglyceride had disappeared. The principal difficulty in experiments bearing on these points is the occurrence of exchange of these labeled moieties in chylomicrons with other plasma lipoproteins (136, 189, 306), which may make net transfer or 'conversion' more apparent than real. It is also not possible to exclude at this time the possibility that some lipolysis occurs at tissue cell interfaces with removal of triglyceride and return of lipoprotein 'skeletons' of higher density than the chylomicrons to the blood. This is particularly important in view of the findings of Jones *et al.* (203) in humans during fat absorption (see below).

A great deal of literature has accumulated suggesting that, in certain disease states, or even at different stages of life in man, the removal of ingested fat may be different. Only a few of these studies have been so designed that they have been able to exclude the important factor of absorption as accounting for some of the differences noted. Because they are concerned with possible pathological variations in a poorly understood physiological process, it will not be attempted to cover these investigations here. The only physiological influence upon chylomicron removal and metabolism which will be mentioned is that of the effect of nutritional state on removal of dietary fat from the blood. In a recent series of papers, Albrink, Fitzgerald and Man (7, 8, 11) have demonstrated that the feeding of glucose or administration of glucagon decreases the degree of alimentary lipemia after a standard fat meal in humans. As they have pointed out, it is impossible completely to eliminate the effects of these agents on rate of absorption, but the glucagon experiments make it unlikely that this factor accounts for the changes they observe. They have also observed a rise in serum triglycerides when epinephrine was given during fat absorption. They have concluded that the degree of alimentary lipemia may be decreased by factors promoting glucose utilization and increased by factors inhibiting carbohydrate metabolism, such as fasting and epinephrine. Whether epinephrine has a real effect on removal of dietary fat from the blood would seem to require more proof than has yet been presented, and it is not necessarily through any effect on carbohydrate metabolism that epinephrine might be acting. Other studies, employing more direct approaches in animals, have been reported which support an effect of carbohydrate metabolism on the removal and distribution of exogenous fat. Bragdon and Gordon (67) noted that carbohydrate feeding in rats so changed the distribution of injected chylomicron fatty acids that they appeared in significant quantities in the adipose tissue. Bragdon, Havel and Gordon (69) found no difference in the removal rates of chylomicrons injected into fasting and carbohydrate-fed rats. On the other hand, Waddell and Geyer (353) have found that the removal of injected fat emulsions was delayed in diabetic rats and restored toward normal with insulin. Fredrickson, McCollester and Ono (137), studying two dogs, found that a high carbohydrate diet and glucose administration was accompanied in each dog by a slight increase in the rate at which chylomicrons were removed. The significance of such small sampling will have to be established by

more experiments of this type. As might be expected from prior work on the sparing of fatty acid oxidation by carbohydrate (241), carbohydrate-loading decreases the oxidation of labeled chylomicron fatty acids in rats (62) and in dogs (137). As measured by the maximum radioactivity of the plasma UFA after carbohydrate feeding in dogs (137), the fractional rate of hydrolysis and return of triglyceride fatty acids to the blood as UFA appears to be decreased.

Transport of Endogenous Fatty Acid as Glyceride

Triglycerides, and possibly significant levels of lower glycerides (82), are present in plasma in the fasting state; and increased concentrations of glycerides, and of chylomicrons and Low Density lipoproteins which are the principal vehicles for glyceride transport, may be observed under a variety of conditions other than during the absorption of dietary fat. The mere presence of glycerides in the plasma during these times does not, of course, imply that they are participating in significant net transport of endogenous fatty acids; and all of the previous assumptions that movement of fatty acids between such tissues as the liver and adipose depots involves transport as glyceride must be reconsidered in the light of what is now known about the remarkable capacity of the unesterified fatty acids of the blood for net transfer. A number of experiments employing isotopes have been done, which indicate, however, that if fatty acid esters are important in transport, the triglycerides are most probably involved. The tentative conclusions, discussed earlier, that unesterified fatty acid turnover in the plasma probably does not supply all of the non-carbohydrate calories in the fasting state, also direct attention to the net transport potential of the glycerides. In earlier experiments, Pihl and Bloch (286) measured the rates of incorporation of labeled acetate into non-phospholipid and phospholipid fatty acids in the rat, and concluded that non-phospholipid fats were more likely to represent a major vehicle for fat transport. Harper, Neal and Hlavacek (182) and Lipsky *et al.* (238) performed similar experiments in the dog and man and arrived at the same conclusions. More recently, Lipsky and co-workers (239) refined these experiments to include chromatographic separation of the non-phospholipid esters. At the time of maximum incorporation of the labeled precursor, the specific activity of the triglyceride fatty acids was 2–10 times that of the phospholipid fatty acids and 20 times that of the sterol ester fatty acids. The turnover of the triglyceride fatty acids appeared to be much greater than that in the other esters, although they did not attempt to derive a turnover time from the complex curves which they obtained. Bates (22) recently reported that, in the fasting dog, the turnover rate of the plasma triglyceride fatty acids was approximately 1.5 grams per hour. The full details of her experiments, which involved cross-circulation, have not yet been published; but her estimate is about half of the plasma turnover of unesterified fatty acids measured in the fasting dog by Fredrickson, McCollester and Ono (137). In older work, labeled fatty acid precursors or labeled fatty acids themselves have been administered (21, 296, 339, 340) and changes in the specific activity of the adipose depots and the liver followed in attempts to establish the likelihood of transfer of fatty acids from liver to adipose tissues and vice versa. In one example of such experiments, Barrett, Best and Ridout (21) fed deuterium-labeled linoleic acid to mice and subsequently measured changes in deuterium content of the fatty acids of the liver and adipose tissues under a variety of conditions. When they fed a high carbohydrate diet, low in lipotropic factors, which caused the animals to develop fatty livers, the deuterium content of the liver fatty acids fell greatly and that of the depot fatty acids changed much less. They felt

that one possible interpretation of their results was the "... transfer of large amounts of fat (presumably made from carbohydrate) from the liver to the depots." Other work indicates, however, that under the condition of carbohydrate excess the adipose tissue itself may synthesize more fatty acids than are formed in the liver. Shapiro and Wertheimer (326) found that D_2O was incorporated into fatty acids of adipose tissue upon *in vitro* incubation. This has subsequently been confirmed by others using different labeled precursors, including glucose. Favarger and Gerlach (124) compared the incorporation of labeled acetate and uniformly labeled glucose into the fatty acids of various tissues as early as 3 minutes after the injection of the substrates. They found higher specific activities in the mesenteric fat than in the liver, and very low specific activity in the blood fatty acids. Shapiro (324) has recently reviewed most of the literature relative to fatty acid synthesis by the adipose tissue, and concludes that there is no doubt that fat formation can proceed independently of the liver, and that the bulk of newly formed fatty acids in the adipose tissues may arise in the adipose and not elsewhere. These considerations make it difficult to interpret the increases in triglyceride which may be seen during high carbohydrate intake. Diets containing excess calories as either carbohydrate or protein were shown by Walker and his associates (359) to elevate S_f 12–400 lipoproteins in man. Bragdon (61) observed that ground squirrels eating low-fat diets in caloric excess developed striking increases in triglyceride compared to the levels present during hibernation. Isocaloric diets extremely low in fat, but high in carbohydrate, also have been shown to elevate serum triglycerides (5) and S_f 20–400 lipoproteins (267). If it is assumed that these high levels of triglyceride represent increased net transport of fatty acids, there are two reasons why the direction of transport might still be considered to be generally in the direction from liver to adipose depots. One is that any excess of fatty acids synthesized in the liver is not likely to be transferred to adipose depots as unesterified fatty acid. Carbohydrate loading decreases total plasma unesterified fatty acid turnover (134, 227) and the direction of any remaining turnover vector is still away from the adipose depots (67). The other is the observation that chylomicron triglyceride is preferentially shunted to adipose tissue when excess carbohydrate is available (67).

Even more striking than the changes in carbohydrate excess are those seen in the presence of defective carbohydrate utilization or lack of adequate available carbohydrate, such as in fasting. Man and Albrink (248) have recently reviewed some of the literature regarding serum lipid changes associated with altered carbohydrate metabolism. It is now well known that diabetic acidosis and glycogen storage disease are associated with marked elevations in serum lipids, particularly the glycerides (36, 79, 183, 250) and Low Density lipoproteins (214, 350). Hyperlipemia can occur in diabetics even on a low-fat diet (36). Delayed removal of exogenous fat in diabetes has also been observed by Waddell and Geyer (353), who injected fat emulsions into diabetic dogs and noted a delay in the removal of lipid from the blood. Insulin increased the rate of clearing to that in normal animals. Chernick and Scow (86) have observed that within 2 hours after pancreatectomy in fasting rats, the blood lipids are elevated even before the blood sugar has reached diabetic levels. At 24 hours this increase is 70 per cent triglyceride. Insulin at this time reduces the non-phospholipid fats to normal fasting concentrations within 3 hours. If the animals are depleted of body fat stores prior to pancreatectomy, the blood triglycerides do not rise.

The changes in blood fats produced by fasting are similar to those associated with uncontrolled diabetes, although usually lesser in degree. Very early work in animals indicated that total lipids, particularly glycerides, will rise if the fast is long

enough. Bloor (45) noted that dogs, which are variable in response to fasting, would always show a rise in blood fats if they were heavily pre-fed fats before the period of fasting. Rony and Ching showed that dogs seemed to have a delay in removing alimentary lipemia when first fed after a prolonged fast (307). This delay was removed when very small quantities of glucose were also fed. Rony *et al.* (308) also reported phlorizin poisoning enhanced fasting lipemia in dogs. Kartin, Man, Winkler, and Peters (205) using more specific analytical methods, could not find consistent changes in plasma lipids of dogs fasted 4–14 days. In man and the monkey, prolonged fasting (2–6 days) has been reported to result in more increase in phospholipids and cholesterol than in triglycerides (205), and these changes have been reversed by small quantities of carbohydrate, enough to reduce accompanying ketosis. Rubin and Aladjem (310) studied changes in lipoproteins during fasting. After 24 hours there were variable changes in the S_f 12–400 lipoproteins; after 96 hours there were increases in both the S_f 12–400 and 0–12 classes, with no changes in the High Density lipoproteins. They found no change in lipoproteins after giving 1 gram per kilo of sucrose to their subjects, although Havel (187) points out that this may simply have represented too little carbohydrate for an effect. Havel (185) found variable changes in the $S_f >$ 10 classes after a 15-hour fast, but he did note that the concentrations of these lipoproteins fell when 400 grams of glucose were fed. The concentration of the High Density lipoproteins also rose during fasting and was lowered by glucose. Diurnal variations in total lipids (59) and lipoproteins (85) have also been studied, and, while the triglycerides show the greatest variation, any such changes must be interpreted as the resultant of the effects of both exogenous fatty acids and carbohydrate.

If it is assumed that the effects of poor carbohydrate utilization and fasting have the same origin, and that the characteristic increases in blood glycerides may represent a brisk net turnover of fatty acids, the mechanism involved is an intriguing and poorly understood one. First, it is likely that the stimulus is not necessarily lack of calories but of carbohydrate utilization, a possible difference pointed out many years ago by Peters (281). Secondly, it is almost certain that the source of the excess esterified fatty acids in the blood is the adipose tissue, as the experiments of Bloor (45) and Chernick and Scow (86), for example, would indicate. This does not absolutely mean that the fatty acids have to leave the adipose as triglyceride, although this seems likely. It is theoretically feasible, from what we know of the tremendous outpouring of unesterified fatty acids from adipose under these circumstances, that some of these might be made into triglycerides elsewhere and returned to the blood. Whatever the origin of the triglycerides, it is apparent that the adipose is reluctant to take them back in the absence of some critical amount of carbohydrate utilization. The delayed removal of fat emulsions in diabetic animals (353), the apparent effects of glucagon and glucose in the fat-fed human (7, 8, 11, 248), and the evidence in the rat that chylomicron fat appears in adipose tissue only when adequate carbohydrate is available (67), all bear most directly on this point. If carbohydrate deprivation does cause release of triglyceride, it might be postulated that this represents an auxiliary mechanism for transporting fatty acids to meet caloric demands. In this regard, we have measured the plasma turnover of unesterified fatty acids in several patients with mild diabetic acidosis (135), using the isotopic method (133, 134), and have found plasma turnover to be greater than the equivalent of 6000 calories per day. These calculations do not indicate how much of this plasma turnover is being utilized for caloric purposes, but it is obvious that this mechanism alone can move enough fatty acids from depots to the liver to compensate for carbohydrate lack, without the

need for net transport as glycerides or other esters. Again, it should be stressed that there is no evidence that any significant amounts of these unesterified fatty acids can get back into the adipose tissue, even under normal conditions, in the unesterified form (67). Triglyceride transport may normally be important for this purpose, and an overloading of this pathway, coupled with a block in triglyceride uptake at the adipose depots could account for 'diabetic lipemia.' The quantity of pure speculation in this formulation indicates the amount of work remaining to be done on this problem.

Several other agents which have demonstrable effects on the level of plasma triglycerides include the adrenocortical hormones and epinephrine. The literature concerning hormonal effects on blood lipids has recently been reviewed by Adlersberg (1). The effects of cortisone, hydrocortisone, or ACTH appear to be variable and species-dependent. While rabbits given these hormones develop lactescent sera and increases in all plasma lipids, particularly triglycerides (3), humans apparently do not (2). Lipemia has been induced by cortisone in a hypophysectomized, diabetic primate (153). The reported effects of epinephrine on fat metabolism, recently reviewed by Ellis (112), are variable, but increases in plasma triglycerides have been obtained in animals and humans (105, 106, 204). A possible effect on the clearing of alimentary lipemia (248) has already been mentioned, and the apparent effect on endogenous triglyceride metabolism is probably part of the same mechanism. Anterior pituitary extract (21, 340) has been reported to mobilize depot fatty acids to the liver. These reports do not contain data on plasma triglycerides. Seifter and Baeder (319–321) have reported the isolation of a lipid mobilizing factor from the plasma of animals administered cortisone and from the posterior pituitary of hogs. This material, which they have prepared as a crystalline peptide, produces elevated levels of plasma triglycerides and lesser elevations in cholesterol and phospholipid, when given to animals suffering from experimental liver damage. This effect also was produced in hepatectomized animals and they conclude that this factor mobilizes fatty acids, principally as triglyceride, from adipose depots. The relationship of this factor to the adrenal hormones or those of the anterior pituitary is not yet clear. Nor is it clear whether the effects of these agents on plasma triglyceride levels and movement of depot fat are all related to an effect on carbohydrate metabolism or due to some other independent mechanism. Presumably the hyperlipemia of pregnancy (282) is related to these poorly understood humoral mechanisms.

The *in vivo* action of lipoprotein lipase inhibitors might theoretically be used to determine whether turnover of endogenous triglyceride is occurring, provided an intact lipoprotein lipase mechanism is required for such turnover. Brown (72, 74) failed to produce lipemia in fasting rats by injecting protamine, but Bragdon and Havel (68) were able to do so with comparable doses. Carbohydrate feeding abolished this protamine effect (69). Spitzer (332) failed to get lipemia in fasting dogs and Brown (73) found no effect on the lipemia of pregnant rats. Thus, results with protamine are variable, but the positive results (68) are compatible with net transport of fatty acids by triglyceride, which is decreased by presence of adequate carbohydrate, and which requires lipoprotein lipase activity to function normally. Other examples of induced lipemia, especially hyperglyceridemia, which probably represent an increase in plasma lipid of endogenous origin include that following repeated hemorrhage (48) or injection of toluidine blue (194) or surface-active agents such as Triton A20 or Tween 80 (207).

Several experimental conditions that result in low triglycerides in the plasma

suggest other factors that may regulate fatty acid transport by glycerides or other esters. Stetten and Salcedo (340) concluded that choline-deficient animals could degrade fatty acids normally and synthesize an excess in the liver, but that there was deficiency in transport of these acids to the adipose depots. Olson (270) has found that choline deficiency in rats results in low plasma triglycerides with lesser decreases in cholesterol and phospholipid. The major change in the lipoprotein pattern is a definite fall in the Low Density lipoproteins. He also states that protein deficiency in man, dog and the monkey produces relative hypolipemia. Ethionine has also been shown to reduce serum lipids and lipoproteins in dogs by Feinberg *et al.* (126), and these authors felt that the effect on lipid metabolism might have been secondary to impaired protein synthesis. Wang *et al.* (360) attempted to use ethionine to produce pancreatitis (120, 159) in order to further study the relationship of pancreatitis to hypertriglyceridemia (212). They obtained a fall in all serum lipids in dogs, but a transient rise in triglycerides preceded the fall in the rabbit given ethionine. The effects of choline deficiency and ethionine administration are very complex, the latter also involving an effect on carbohydrate metabolism and a relationship to the adrenal cortex (367). However, it is conceivable that they represent examples of deficient transport of fatty acids because of vehicular failure, i.e., deficient production of lipoprotein protein or phospholipid, or even cholesterol, or a breakdown in the proper assemblage of these components into lipoprotein molecules. This is destined to remain speculation at least until such fundamental information as the site of lipoprotein formation itself is definitely established. If one is permitted the theory of 'vehicular lack,' it requires little further license to advance the possibility that 'vehicular excess' may be responsible for some of the rises in plasma triglycerides observed in hypercholesterolemic conditions. When cholesterol is fed to rabbits, for example, it has been repeatedly demonstrated that they rapidly develop great excesses in Low Density lipoprotein, with consequent rises in triglyceride. Thus, it is possible that under some conditions in which more Low Density lipoprotein is required for transporting extra cholesterol or phospholipid, triglyceride levels could be passively elevated.

It is apparent that our knowledge of the role of triglyceride in the net transport of endogenous fatty acids is quite limited. It is especially important that more work be done on the caloric contribution of the turnover of plasma triglycerides. This is related to the problem of determining the relationship of plasma unesterified fatty acid turnover to actual utilization. Neither of these problems is easy to solve experimentally in the whole animal. For single organs, such as the heart (167), it appears that unesterified fatty acid transport is of greater importance. For the present, it will be necessary to establish for each example of elevated triglyceride or Low Density lipoprotein levels in the blood the difference between increased concentrations and increased net transport of fatty acids in this form.

Lipoprotein Interconversions

A most important consideration is the manner in which the various lipoproteins may be functionally interrelated in transporting fatty acids. This point is inextricably bound to the question of interconvertibility of lipoproteins, and with the possibility, proposed recently (234), that chylomicrons and other very low density molecules can be shorn of lipid in the normal course of their metabolism and yield Low Density lipoproteins of lower S_f values, or even High Density lipoproteins. The concept of 'interconversion' arose first out of the observations of changes in lipoproteins which occur *in vivo* after injection of heparin, or *in vitro* after incubation of lipoproteins

with post-heparin plasma (13, 60, 85, 173, 230, 233, 236). The first such studies reported were those of Graham *et al.* (173), who found in the intact rabbit or human that heparin induced a fall in the S_f 20–400 lipoproteins and a concomitant rise in the S_f 12–20 class, which was followed by decline of the latter class to normal concentrations within a few hours. They also observed serial rise and fall in the concentrations of Low Density classes down to S_f 6. The increase in S_f 0–20 lipoproteins did not appear to account for all of the fall in S_f 20–400 lipoproteins which was observed. They obtained similar findings *in vitro*, as did Anfinsen, Boyle and Brown (13), and Lindgren, Nichols and Freeman (236), who felt that the S_f 0–20 lipoproteins 'produced' by such 'interconversion' *in vitro* were similar (on the basis of density) to those of that class occurring physiologically. They felt that the falls in S_f 20–400 lipoproteins through lipoprotein lipase activity may have been accompanied by some increase in High Density lipoproteins. *In vitro* production of High Density molecules had previously been reported by Boyle, Bragdon and Brown (60), but in attempts to repeat this work, using preparative ultracentrifuge fractions, Bradgon and Havel found only rises in the phospholipid in the High Density fraction (64). Therefore, the possible production of High Density lipoproteins during this *in vitro* process needs further documentation. Robinson and French (299) have suggested that during hydrolysis *in vitro*, complexes of cholesterol with fatty acids might form which simulate the ultracentrifugal properties of High Density lipoproteins. It should be pointed out here that changes in electrophoretic mobility of lipoproteins in post-heparin plasma (122, 268, 309), sometimes interpreted as lipoprotein conversion, have been shown to be due to the binding of unesterified fatty acid released by hydrolysis to the lipoproteins and consequent change in their charge (166, 225, 258). There seems little reason to doubt that when sufficient lipoprotein lipase activity is present in the blood, Low Density lipoproteins may be 'converted' to other Low Density lipoproteins of lower S_f value, primarily by loss of triglycerides through hydrolysis. Despite the demonstration of very low titers of lipoprotein lipase activity in human (116) and rat (201) plasma in the absence of exogenous heparin, it appears (149, 187) that there is not sufficient plasma activity to cause this phenomenon normally to occur intravascularly without the intervention of at least some tissue surface. In another species, the dog, Havel and Fredrickson (191) could find no evidence of hydrolysis of labeled chylomicrons during *in vitro* incubation in dog plasma in the absence of heparin.

Similar 'interconversions' have been reported *in vivo*, however, in the absence of exogenously administered heparin. All of these have been characterized by falls, first in the lightest classes of Low Density lipoproteins (S_f 100–400), with serial rise and then decline in the concentrations of the more dense classes, in a step-wise fashion. Pierce and Bloom (285) and Hewitt *et al.* (194) observed these changes during the disappearance of cortisone- and radiation-induced lipemia in rabbits. Tuller *et al.* (350) and Kolb, de Lalla and Gofman (214) found similar events occurring after treatment of lipemic diabetics in acidosis. The latter authors also studied High Density lipoprotein changes during these transformations, and noted that the previously elevated HDL_1 concentrations fell, but HDL_2 and HDL_3 concentrations did not change with the fall in the Low Density classes. Pierce (284) injected intravenously Low Density lipoproteins of various S_f classes and observed the above-mentioned serial changes in all S_f classes below those injected. These changes included S_f 5–15 molecules, although the elevated concentrations of this class took days, instead of hours, to return to normal.

Bragdon, Boyle and Havel (66) reported that, when human lipoprotein fractions

were injected intravenously into rats, there occurred rises in concentrations of both Low and High Density lipoproteins. In fact, under these rather unphysiological conditions, they were able to produce lipemia by injection of High Density lipoproteins. Except for these experiments, conversion of lipoproteins has so far been demonstrated to be a unidirectional process, from lipoproteins of lower density to those of higher density (284). These experiments (66) also contain the only evidence of conversion of lipoproteins of $S_f > 400$ to those of lesser flotation rate.

Jones *et al.* (203) reported in 1951 that administration of high fat meals to humans resulted in transient increases in molecular classes 'above S_f 60' in the plasma, followed by stepwise serial changes in the concentrations of lipoproteins down to S_f 30. They found no change in the concentrations of lipoproteins of S_f 30 to S_f 4. No experimental data accompanied the report of these important observations, and little other work has been reported in which serial changes in lipoprotein concentrations after fat feeding have been studied. Havel (184), using preparative ultracentrifuge fractions to measure changes in concentrations by chemical analysis, found a rise and much later decline in cholesterol and phospholipid, especially in the major High Density lipoproteins after fat feeding, which he felt was not inconsistent with step-wise degradation of Low Density molecules even down to High Density lipoproteins. In the dog (191) the injection of labeled chylomicrons is accompanied early by the appearance of only small amounts of radioactivity in the other Low or High Density fractions. This animal, however, normally has very low concentrations of Low Density lipoproteins and it is conceivable that some 'interconversion' may take place with a very rapid turnover of the Low Density lipoprotein triglyceride. The experiments in the dog (191) and those in rats by Bergström *et al.* (28) have already been mentioned as failing to support the hypothesis that chylomicrons are sequentially degraded while in the blood, or that any large fraction of Low Density lipoproteins of lower S_f values are derived from them during fat absorption in these animals. Finally, Gitlin *et al.* (156) have observed that when lipoproteins of S_f 10–100 are tagged in the protein moiety with I^{131} and injected into humans, the radioactivity appears shortly in the molecules of S_f 3–9. As Pierce had observed in different experiments (284) there appeared to be no conversion of lipoproteins of low S_f values to those of higher S_f values. Gitlin *et al.* (156) found that significant conversion within the Low Density classes did not occur on incubation *in vitro*, and that there was no significant transfer of radioactivity either *in vitro* or *in vivo*, to High Density lipoproteins. Avigan, Eder and Steinberg (18) also observed no interconversion between Low and High Density lipoproteins *in vivo*. In the studies done in humans (156) it was not possible to make allowance for the possibility that since S_f 0–100 lipoproteins may contain several peptide chains (303, 328), only one of these chains may have been labeled with the I^{131}. It would be important to eliminate any possible heterogeneity of the distribution of the protein label before concluding that all Low Density lipoproteins within a given flotation class are convertible, or that no Low Density lipoprotein protein may ever appear in the High Density fractions. Since nothing is known about possible heterogeneity within the Low Density molecules of the rabbit, the results obtained by *in vivo* labeling of the lipoprotein proteins with C^{14} (18) do not help clarify this question in regard to the human.

In a recent publication, Lindgren *et al.* (234) have advanced the theory that "as a result of serum lipoprotein lipase activity," lipoprotein transformations occur throughout the entire S_f 20–10^5 range of lipoprotein complexes (in man). They have also compared the lipid compositions of various classes throughout the full density

spectrum with those theoretically obtainable by removing triglyceride alone from the molecules. They conclude that chylomicrons and Low Density lipoproteins down to S_f 20 may be cores of lipid surrounded by HDL_2 and HDL_3 High Density lipoproteins, and that the S_f 0–20 Low Density lipoproteins consist of HDL_1 lipoprotein surrounding a core of residual triglyceride and 'contaminant' phospholipid and cholesterol carried in with the chylomicrons. This scheme of transformations is constructed almost entirely of arithmetical calculations, and does not make reference to all other available information. In light of what is known today, such a unifying hypothesis can neither be strictly proved or disproved, but to make it tenable much effort must be made, particularly to reconcile it with what is known about the nature and behavior of the peptide portions of the lipoprotein molecules.

Our present knowledge of the chemical nature of the protein portions of the major lipoprotein classes in the human has been previously discussed, and the principal N-terminal residues are presented in figure 1. It is apparent that the N-aspartic acid-C-threonine (328) proteins of the HDL_2 and HDL_3 molecules are quite different from the N-glutamic acid-C-serine (19, 71, 303, 328) peptide chains predominating in the class S_f 0–20 or slightly higher. In addition to these peptide chains, it is clear from the work of Shore (328) and Rodbell (303) that polypeptides with N-terminal serine and threonine begin to predominate as the Low Density molecules assume higher flotation rates. Both lymph and plasma chylomicrons appear to be associated with more than one protein (303–306). Although the largest fraction of the chylomicron protein is identical to the protein found in the major High Density lipoproteins (303–306), it is not yet known whether this protein is present, in or on the surface of chylomicrons, merely as a polypeptide, or as the full High Density lipoprotein complex. This protein or lipoprotein is in equilibrium with the High Density lipoproteins in plasma or lymph (305–306), and in this sense at least, the chylomicrons and major High Density lipoproteins are related. At least one other protein is consistently isolated in chylomicrons (303–306), and this may contain N-terminal serine or threonine, common to some of the other very low density lipoproteins. Specific information concerning the nature of the HDL_1 lipoprotein protein moiety is not yet available. This is quite pertinent to the possible presence of this lipoprotein in some molecules of the Low Density classes. Then, there are the above-mentioned studies (18, 156) in which the behavior of tagged lipoprotein proteins has been observed. The failure so far to demonstrate transfer of labeled protein from S_f 10–100 molecules to the High Density lipoproteins (156) is particularly inconsistent with the theory that, by intravascular transformation, very low density lipoproteins lose their triglyceride, leaving only a residue consisting of the major High Density lipoproteins (234). A possible reason why the labeled experiments of Gitlin *et al.* (156) might have failed to detect such a transformation has just been discussed.

On the basis of present knowledge it is safe to assume the following about lipoprotein interconversions: that conversion of Low Density lipoprotein molecules of $S_f < 400$ to others of lower flotation rate occurs; that this conversion process depends largely upon loss of triglyceride, which is catalyzed by lipoprotein lipase or similar enzymes; that this conversion might occur in the blood stream under certain physiological conditions, particularly in man, but the intervention of certain tissue sites is probably essential; that the presence of Low Density lipoproteins having the same flotation rate but different peptide chains is a distinct possibility, implying that all Low Density lipoproteins are not necessarily 'generically' related; that conversion of chylomicrons and lipoproteins of $S_f > 400$ to Low Density molecules of

$S_f < 400$ remains to be proved; and, finally, that chylomicrons are related to High Density lipoproteins insofar as one of the polypeptides associated with these particles is identical to that common to the major High Density lipoproteins. With increasing knowledge about the peptide portions of the lipoproteins, we may expect much clarification of these rather tentative relationships, but no unifying hypothesis yet presented is consonant with all of the experimental facts.

Role of Phospholipids and Sterol Esters in Fatty Acid Transport

Phospholipids. Experimental evidence that implicates phospholipids in various processes of absorption and mobilization of fatty acids has accumulated from time to time over the past several decades. In the majority of the older experiments, the evidence was indirect, and sufficed merely to show that alterations in phospholipid metabolism occurred under conditions in which fatty acid transport was active. Thus, Sinclair (330) observed changes in the iodine number, but no alteration in the total amount, of phospholipid fatty acids in the intestinal mucosa of fat-fed cats. When he fed cod-liver oil or coconut oil, the iodine value of these intestinal tissue fatty acids changed toward the iodine value of the fat administered. Favarger (123) performed similar experiments in dogs, giving glycerides of elaidic acid, and found incorporation of elaidic acid into the lecithin fraction, in particular. This incorporation was maximal in the most superficial cell layers. Schmidt-Neilsen (318), in early experiments with P^{32}, noted an increase in radiophosphorus incorporation into the intestinal phospholipids when sodium oleate was given. In his experiments, each rat served as its own control, since the oleate was given intraluminally into closed intestinal loops; the treated loops utilized more of a systemic dose of P^{32} than did control loops similarly handled, but without oleate. These various results have been felt to support the hypothesis, first formulated by Sinclair (330), that the course of fat absorption in the intestine involved obligate conversion of free fatty acids arising from lipolysis in the lumen to phospholipids, and thence to triglycerides.

Zilversmit, Chaikoff and Entenman (369) made similar observations with P^{32}-labeling of intestinal phospholipids in rats, but failed to find a corresponding phenomenon in dogs. They felt, however, that the rate of turnover of phospholipid in fat-fed rats was not adequate to support Sinclair's hypothesis. In their introductory section, they remark that it is only natural that labeled fatty acids fed to experimental animals are found in intestinal phospholipids, inasmuch as the latter are undergoing continual breakdown and resynthesis, and the process of resynthesis could utilize exogenous fatty acids during absorptive periods.

This suggestion can be refined in view of recent investigations into the metabolism of phospholipids in tissue slices and cell-free systems. Hokin and Hokin (196) report on a series of investigations in which it was shown that the phosphorus and choline, but not the glycerol, of the phospholipids of glandular tissue (pancreas) turn over more rapidly when the secretory activity of the tissue is stimulated. This implies degradation of phospholipid to diglyceride and other fragments, accompanied by resynthesis from that stage. Recent advances in the study of the routes of synthesis of phospholipids and of triglycerides, reviewed by Kennedy (209), have demonstrated the central position of diglycerides, which are converted either into phospholipids or triglycerides by esterification with appropriate activated compounds. If the metabolic activity accompanying absorption of fat in some way causes a rapid turnover of phospholipids within the musocal cell, and if that cell is at the same time synthesizing triglycerides from fatty acids absorbed from the intestine (diglycerides being an

essential intermediate in such synthesis), it is not surprising that the exogenous fatty acids make their appearance to some extent in the tissue phosphatides. This interpretation gains considerable support from the observation of Borgström (53) that the feeding of C^{14}-labeled fatty acids to rats resulted in incorporation of radioactivity into both the phospholipids and triglycerides of the intestinal mucosa, but that the specific activity of the triglycerides always exceeded that of the phosphatides. Thus, Sinclair's hypothesis, though not correct as stated originally, is not absurd, as it now appears that phospholipids are related in a very direct way to diglyceride, which in turn actually is an obligate intermediate in the resynthesis of triglycerides in the intestine.

In spite of the vigorous turnover of tissue phospholipids during fat absorption, it appears that very little of this material actually leaves the intestine in chyle. The phospholipids of chyle and lymph have been shown to be derived in part from the lipoproteins of plasma by Reinhardt, Fishler and Chaikoff (292). Their rate of transfer from plasma to lymph is believed by Borgström and Laurell (57) to be accelerated during fat absorption. There is evidence, however, of the presence in chyle of phospholipids newly formed in the intestine. Bollman and collaborators (49) observed that the thoracic duct lymph of dogs had a higher content of phospholipids, but not of cholesterol, when active fat absorption was occurring. An increased content of lipoprotein derived from plasma could not have produced this disproportionate effect. Borgström (54) and Bloom *et al.* (43) have observed that an appreciable fraction of the radioactivity in thoracic duct lymph of rats fed C^{14} fatty acids is contained in the fatty acids of the phospholipids. The proportion in the phosphatides tended to increase with increasing chain length of the fatty acid used (54). Thus a small, but real, fraction of dietary fatty acids appears in the thoracic duct chyle, and is presented to the plasma, as phospholipid. The further fate of this specific fraction is not known.

The phospholipids of plasma exist exclusively as components of the lipoprotein complexes whose chemistry and metabolism have been discussed earlier. Kunkel and Bearn (220), and Eder (108) have shown that the phospholipids are not firmly attached to the lipoprotein molecule, but that exchange of phosphatides between lipoprotein species may occur *in vitro*. Zilversmit *et al.* (370) first utilized P^{32} to measure the turnover rate of plasma phospholipids in the dog. Weinman *et al.* (362) injected plasma containing phospholipids labeled in both the phosphorus and fatty acid moieties into recipient dogs. The turnover times obtained for both moieties were comparable, and were not higher than 0.17 millimoles phospholipids per hour. In another series of studies, workers at the University of California have shown that plasma phospholipids are synthesized and degraded almost exclusively by the liver. Fishler *et al.* (128) reported that removal of the liver from dogs virtually abolished the incorporation of P^{32} into plasma phospholipids but had little effect on the phospholipids of gut and kidney. Entenman *et al.* (117) showed that the degradation of P^{32}-labeled lipoproteins, taken from a donor dog to whom inorganic P^{32} had been given, was dependent on the presence of the liver in the recipient animal. Later, Goldman *et al.* (161) administered C^{14} labeled fatty acids to dogs, and found that removal of the liver prevented incorporation of these tracer substances into plasma phospholipids without impairing incorporation into phosphatides of other tissues. Recently, Lipsky and collaborators (239) have investigated the rate of incorporation of carbon-14 given as acetate into the fatty acids of the plasma lipids of man. They find much more rapid turnover in triglycerides than in phospholipids, and suggest that the former is a metabolic precursor of the latter.

There is no experimental evidence at present which proves an important role of plasma phospholipid in the net transport of fatty acids, except as an important component of the lipoprotein vehicles which may be involved in the transport of triglyceride. This is not to imply, of course, that phospholipids in tissues (other than the intestine) may not have some important function in mobilizing fatty acids. The rises in phospholipid which occur in a number of conditions, including diabetic acidosis (36, 250) or with fasting (205), must presently be interpreted as an expression of one of these functions. The report by Peterson (283) that the rise in phospholipid seen in starvation in man consists almost entirely of sphingomyelin is in line with the finding of a higher proportion of sphingomyelin in the Low Density lipoproteins (336), the predominant carriers of triglyceride. There seems no new experimental basis, therefore, for disagreement with Beveridge (30), who, in his review several years ago of the biologic functions of phospholipids, discounted their importance as direct carriers of fatty acids.

Sterol Ester. At the present time there is no evidence which indicates a role which cholesterol ester may play in net transport of fatty acids. Considering the known plasma turnover of fatty acids in the unesterified form, and the possible turnover of fatty acids in the form of triglyceride, it is almost certain that cholesterol esters do not have a significant role in transporting fatty acids from one tissue to another for the purpose of meeting caloric requirements. The very slow rate of incorporation of fatty acids into cholesterol esters in the intestine indicates the sterols are unimportant in lymphatic transport of exogenous fatty acids. The findings by the workers in Lund that linoleic acid (38) and oleic acid (25, 38) were both carried in the thoracic duct lymph as triglyceride are especially significant. Since sterol esters, in man at least, appear to contain largely these two fatty acids (239), the possibility of certain specific acids being preferentially transported in lymph as sterol ester also appears to be eliminated. The findings by Lipsky *et al.* (239) of the very slow rate of turnover of the sterol fatty acids, judged by the rates of incorporation of acetate into the fatty acids of the esters found in human plasma, also indicate a minor role for cholesterol in net transport of endogenously synthesized fatty acids. However, linoleic acid, which is an important sterol ester fatty acid, as well as other possible 'essential' fatty acids, is not labeled adequately by these *in vivo* synthesis experiments, and it is impossible to exclude the possibility that sterol ester might be a transport medium of significance within the plasma for certain of these specific acids. These discoveries await further application of such techniques as vapor phase chromatography to kinetic studies with labeled fatty acids. While proof of any important function of sterol ester in net transport is lacking, it will not be remiss to mention the important part they play in the structure of lipoproteins. The presence of cholesterol in even the lightest and most triglyceride-laden of the lipoproteins, suggests, as in the case with phospholipid, a more static vehicular role for cholesterol in fatty acid transport.

Conclusion

It is particularly encouraging that many of the techniques needed to attack some of the pressing problems in fatty acid transport have recently become available and are rapidly coming into wider use. The application of two of these techniques alone, the improved chromatography of fatty acids and the available methods for protein identification, can be expected to clarify greatly the nature of the transport of specific fatty acids and the interplay of the lipoprotein molecules in these processes. Isotopic techniques, which have been so successful in unraveling the synthesis of

simpler molecules, can be turned to the synthesis of more complex macromolecules like the lipoproteins, and more information on the kinetics of fatty acid transport should be obtainable with even presently available techniques. Investigation of a number of other problems has only begun. Awaiting attention at the capillary endothelium is the manner by which lipids and lipoproteins manage so easily their escape and reentry. A fertile area for research lies just beyond the endothelium, in the adipose tissue, the liver and other tissues whose involvement in lipid transport is obvious, but whose manner of participation and control in this endeavor is still obscure. While limited insight has been obtained into some pathological aspects of fatty acid transport, the attack on certain diseases associated with defective lipid transport awaits the strategy which will come from better understanding of the physiological mechanisms.

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